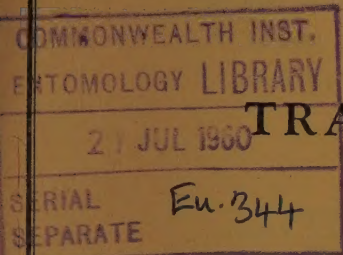


Vol. 112. Pt. 5. Pp. 73-108. 2 Plates. 57 figs. 20th July, 1960



THE
TRANSACTIONS
OF THE
ROYAL
ENTOMOLOGICAL SOCIETY
OF
LONDON

World List abbreviation: *Trans. R. ent. Soc. Lond.*

CONTENTS

	PAGE
X MILNE, D. L. The gall midges (DIPTERA: CECIDOMYIDAE) of clover flower heads	73-108, 2 plates 57 figs.

LONDON:
PUBLISHED BY THE SOCIETY AND
SOLD AT ITS ROOMS, 41, QUEEN'S GATE, S.W.7.

Price 15s. Od.

THE ROYAL ENTOMOLOGICAL SOCIETY OF LONDON

Founded 1833. Incorporated by Royal Charter 1885.

Patron :

HER MAJESTY THE QUEEN

OFFICERS AND COUNCIL FOR THE SESSION, 1960-1961

DR. B. P. UVAROV, C.M.G., F.R.S.,	<i>President</i>
PROFESSOR D. S. BERTRAM	} <i>Vice-Presidents</i>
DR. J. S. KENNEDY	
MR. E. O. PEARSON	
DR. N. E. HICKIN, <i>Treasurer</i>	
DR. PAUL FREEMAN, <i>Secretary</i>	
MR. W. H. POTTS, <i>Editor</i>	

Other Members of Council

MR. R. B. BENSON	MR. H. OLDROYD
MR. R. G. DAVIES	PROFESSOR G. C. VARLEY
DR. A. M. EASTON	DR. N. WALOFF
DR. L. G. HIGGINS	MR. M. J. WAY
DR. B. R. LAURENCE	DR. C. B. WILLIAMS, F.R.S.

MISS E. EVANS, *Registrar*

Finance and House Committee

PROFESSOR D. S. BERTRAM (*Chairman*)

DR. C. G. BUTLER	DR. D. J. LEWIS
MR. J. P. DONCASTER	MR. H. L. G. STROYAN
DR. R. M. GREENSLADE	

Publication and Library Committee

MR. R. G. DAVIES (*Chairman*)

DR. J. D. CARTHY	MR. D. LEATHERDALE
DR. A. M. MASSEE, O.B.E.	MR. G. E. J. NIXON
MR. T. G. HOWARTH	

Conservation (Insect Preservation) Committee

MR. R. B. BENSON (*Chairman*)

DR. B. M. HOBBY	MR. F. T. VALLINS
DR. A. M. MASSEE, O.B.E.	MR. M. J. WAY
MR. N. D. RILEY, C.B.E.	

The Executive Officers of the Society are *ex-officio* members of all Committees.

DELEGATES OF THE SOCIETY TO :

1. **British National Committee for Biology** (Royal Society).
Mr. N. D. Riley, C.B.E., appointed 1955.
Dr. H. E. Hinton, appointed 1958.
2. **Local Committee of Management of Wicken Fen.**
3. **National Trust for Places of Historic Interest or Natural Beauty.**
Mr. N. D. Riley, C.B.E., appointed 1955.
4. **New Forest Association.**
Mr. C. W. Mackworth-Praed, appointed 1948.
5. **New Forest Joint Committee.**
Lt.-Col. F. C. Fraser, appointed 1949.
6. **Field Studies Council.**
Dr. W. H. Thorpe, F.R.S., appointed 1944.
7. **Yorkshire Naturalists' Trust, Ltd.**
Dr. W. D. Hincks, appointed 1946.
8. **The Biological Council.**
Dr. N. E. Hickin, appointed 1952.
9. **The Parliamentary and Scientific Committee.**
Dr. N. E. Hickin, appointed 1953.
10. **British Co-ordinating Committee for Nature Conservation.**
Mr. E. B. Britton, appointed 1954.
11. **Council for Nature.**
Dr. N. E. Hickin, appointed 1958.

THE GALL MIDGES (DIPTERA : CECIDOMYIDAE) OF CLOVER FLOWER-HEADS¹

By D. L. MILNE

(Rothamsted Experimental Station, Harpenden²)

Manuscript received 25th September, 1959

(Read 2nd December, 1959)

With 2 Plates and 57 Text-figures

CONTENTS

	PAGE
I. Introduction	73
II. Seed-destroying species	74
1. The separation of <i>Dasyneura leguminicola</i> Lintner from <i>D. gentneri</i> Pritchard	74
2. <i>Tricholaba barnesi</i> sp. n.	85
III. Predatory species	90
1. <i>Phaenobremia aphidivora</i> Rübsaamen	90
2. <i>Lestodiplosis trifolii</i> Barnes	94
IV. Other species and genera	96
1. <i>Clinodiplosis leguminicola</i> sp. n.	96
2. <i>Giardomyia britannica</i> sp. n.	99
3. <i>Isodiplosis deutera</i> sp. n.	100
4. <i>Brachyneura squamigera</i> Winnertz	102
5. <i>Contarinia</i> sp.	103
V. Key to males, females and larvae of Gall Midges inhabiting clover flower heads	104
VI. Summary	106
VII. Acknowledgments	107
VIII. References	107

I. INTRODUCTION

IN 1879 Lintner described the gall midge *Dasyneura leguminicola* occurring on red clover in the United States. Since then this midge has been recorded as causing seed losses in red clover in most of the countries of Europe, including England and Wales, and a full account of the literature on it up to 1945 is given by Barnes (1946).

In 1953 Pritchard described a new species, *D. gentneri*, the larvae of which were found to cause seed losses in white clover in the United States. He differentiated the males of *D. leguminicola* and *D. gentneri* by characters of the genitalia. A year later Bishop published a paper in the United States on the biology of *D. gentneri*, recording its occurrence on Ladino clover and Alsike clover, and that the female oviposits on open flowers, and not on green clover heads, such as those selected by *D. leguminicola*.

In 1954 Barnes recorded a *Dasyneura* species, bearing a striking resemblance to *D. gentneri*, on white clover in Britain, and also the presence of several other genera of Cecidomyidae on clover.

¹ Part of work approved for the Degree of Doctor of Philosophy in the University of London.

² Now at Central Tobacco Research Station, Rustenburg, S. Africa.

The present investigation was undertaken in order to determine which midge species are present on clover in Britain and, where possible, to establish details of their biologies and distribution. It has revealed that the *Dasyneura* species occurring on white clover in Britain is *D. gentneri*, and, largely because a system of differentiation has been found, not only for males and females but also for larvae of the *Dasyneura* species on clover, it has been possible to draw up illustrated keys for the males, females and larvae of all gall midges occurring on both red and white clover heads in Britain.

The findings of this study are presented in the following order: (1) a description of the midges whose larvae destroy seed, which includes a comparison of *D. leguminicola* and *D. gentneri* and the description of a new species, *Tricholaba barnesi* sp. n.; (2) a description of those midges the larvae of which are predatory; (3) a description of those species of which the larval habits cannot be described with any certainty; and (4) an illustrated key to the larvae and adults of midges occurring on clover heads in Britain.

Illustrations.—All text figures are magnified as follows: male genitalia and sternal spatula, $\times 180$; anal segments, $\times 120$; ovipositors, $\times 105$; wings, $\times 30$.

II. SEED-DESTROYING SPECIES

1. *The Separation of Dasyneura leguminicola* Lintner from *D. gentneri* Pritchard

Introduction

One of the chief aims of this investigation was to determine the identity of the two *Dasyneura* species found on clover in England. Pritchard (1953) recorded *D. gentneri* as occurring on white clover in the United States, but Barnes (1954) describes experiments in which he succeeded in breeding a midge closely resembling *D. gentneri* from red clover in England. He also achieved an apparent intermating between species, when a male *Dasyneura* bred from English white clover mated with a female bred from red clover.

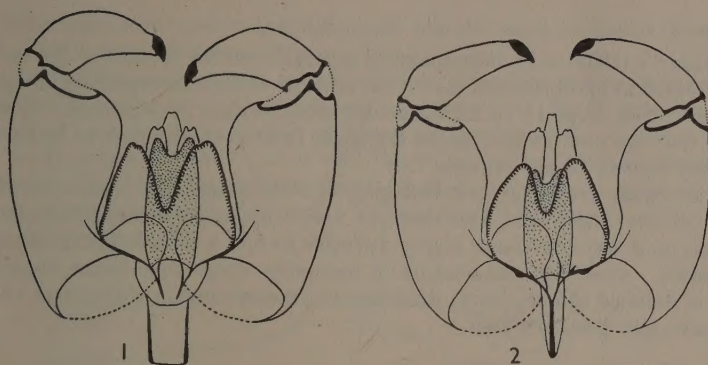
Detailed studies have therefore been made of the morphology and biology of the red and white clover *Dasyneura* midges in Britain, making possible a detailed comparison of the two species. With the aid of biological experiments it has been possible to find methods of separating, morphologically, not only the males but also the females and larvae of both species. Numerous differences in the biologies provide even further confirmation that there are two distinct *Dasyneura* species on clover in Britain. The investigation was made more complex by the fact that the occurrence of *D. gentneri* on red clover as well as on white was confirmed and this gave rise to a mixed population of *Dasyneura* species on red clover. The comparison of the two species is drawn under three headings; (a) morphological differences, (b) biological differences and (c) breeding experiments.

(a) *Morphological differences*

Males

Genitalia.—As described by Pritchard (1953), the main distinction between the males of these two species is in the genitalia. In *Dasyneura leguminicola* there is a projection at the base of the basal clasp segment which is nearly as broad as the ventral plate, lightly sclerotised and regular (fig. 1). In *D. gentneri* the roots of the basal clasp segments unite to form a slender, strongly sclerotised rod, bordered by a somewhat irregular, extremely lightly sclerotised projection (fig. 2). The basal clasp segments are slightly broader distally in *D. leguminicola* than in *D. gentneri*.

Size and colour.—The colour and size of the males vary considerably, but *D. gentneri* is usually paler and smaller than *D. leguminicola*.

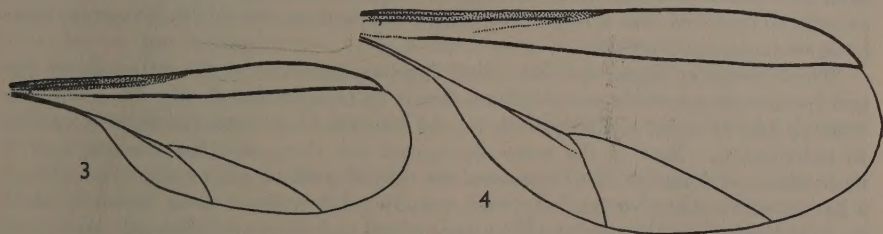


FIGS. 1-2. Male genitalia of: (1) *D. leguminicola*; (2) *D. gentneri*.

Antennae.—The antennae are remarkably similar, but there is usually a greater number of tubercle-based setae on the ventral surface of the third flagellar segment of *D. leguminicola*. A count was made of the antennal segments of a number of specimens of both species with the following results (fusing between segments, particularly the two distal segments, is not uncommon in both species and such specimens were not included in the counts):

Number of segments	Number of individuals	
	<i>D. leguminicola</i>	<i>D. gentneri</i>
2+12	0	8
2+13	5	74
2+14	58	29
2+15	34	0
Total	97	111

The figures show a general overlap for the two species but confirm Pritchard's (1953) observation that the number of antennal segments in *D. gentneri* (most 2 + 13) is generally lower than in *D. leguminicola* (most 2 + 14, and many 2 + 15). The data were analysed according to Yates' (1934) method of analysis of multiple classifications with unequal numbers in the different classes, and by the "t" test it was found that the two sets of figures, representing the two species, were significantly different at the 0.1 per cent. level, thereby confirming the separation of the species.



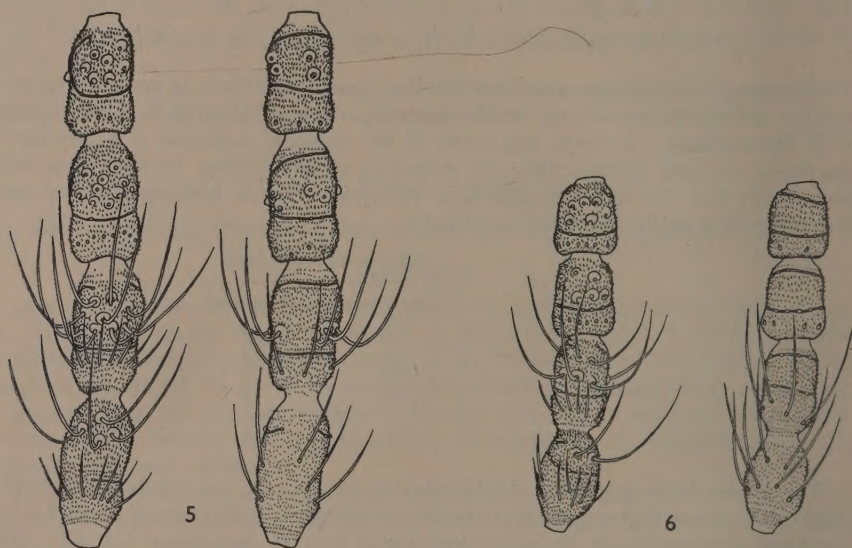
FIGS. 3-4.—Wing of: (3) *D. gentneri*; (4) *D. leguminicola*.

Wings.—The general venation is similar, but in *D. gentneri* (fig. 3) the wing is slightly narrower and smaller and there is never a rudimentary vein proceeding towards the third vein from the point where the first radial vein and costa fuse, as sometimes occurs in *D. leguminicola* (fig. 4).

Females

Pritchard's (1953) only morphological separation of the females of the two species was contained in his statement that "the number of flagellar segments in the females examined varies from 11 to 13 segments, most having 12 segments. It therefore appears that the number of flagellar segments in *D. gentneri* tends to be less than in the closely related *D. leguminicola*".

In view of the overlap in their biologies, with the consequent occurrence of the two species on one plant, and the need to verify apparently successful intermating experiments, it was considered highly desirable to find a reliable means of separating the females. After the examination of numerous microscope slides, together with the use of detailed camera-lucida drawings and measurements, differences were found which have now been analysed.



FIGS. 5-6.—First four flagellar segments (left, ventral; right, dorsal), female, of : (5) *D. leguminicola* ; (6) *D. gentneri*.

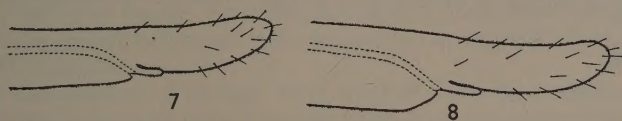
Size and colour.—The female of *D. gentneri* is usually slightly smaller and paler than *D. leguminicola* but the size range overlaps considerably. It was found that, in general, the range was considerably greater in specimens bred in the insectary than in those caught in the field.

Third Flagellar Segment.—The third flagellar segments of the antennae of the two species are separable on two characters. In *D. leguminicola* (fig. 5) it is nearly twice as long as broad medially, with 7 to 16 tubercle-based setae (mean 13, based on 70 individuals). Most of the setae are ventral but there are always some, two or more, situated dorsally. In *D. gentneri* the third flagellar segment (fig. 6) is one and a half times its diameter medially, with 4 to 10 tubercle-based setae (mean 7, based on 70 individuals). Most of the setae are ventral and there are frequently none, and seldom more than one, situated dorsally.

Ratio of Tarsal Segments.—The ratio of the length of the first to the second tarsal segment of the prothoracic legs was calculated in 25 individuals of both species with the following results : *D. gentneri*—second segment 4 to 6 times length of first (mean 5 times, approximately). *D. leguminicola*—second segment 6 to 9 times length of first (mean 7 times, approximately). It would be advisable to carry out a further

series of observations to confirm this difference, but the second segment does appear to be relatively longer in *D. leguminicola*.

Ovipositor.—The terminal part of the extensile ovipositor of these species, when viewed laterally, serves as a valuable means of separation. In *D. leguminicola* the main terminal lobe is approximately as broad as the remainder of the ovipositor (fig. 7). In *D. gentneri*, however, it is only two-thirds as broad as the remainder of the ovipositor (fig. 8). This difference was found to be very constant in a large number of specimens examined, but the importance of viewing the lobe laterally cannot be over-emphasised.



FIGS. 7-8.—Ovipositor, lateral view, of: (7) *D. leguminicola*; (8) *D. gentneri*.

Antennal segments.—A count of antennal segments of both species produced similar, though clearer, results than those given for the males, as shown in the table below:

Number of segments	Number of individuals	
	<i>D. leguminicola</i>	<i>D. gentneri</i>
2+ 9	0	1
2+10	0	0
2+11	0	25
2+12	1	145
2+13	23	70
2+14	61	0
2+15	11	0
Total	96	241

A statistical analysis (as carried out for the males) was made, and the results once again confirmed that the groups, and therefore the species, are significantly different (at the 1 per cent. level).

Larvae

Third instar.—The third instar larvae can be differentiated by the lobes of the sternal spatula, which are present but weakly sclerotised. In *D. leguminicola* the lobes are steeply triangular and quite pointed apically, whereas in *D. gentneri* they are smaller, less steeply triangular and more rounded apically. Certain colour differences are also noticeable at this stage, the fat-bodies of *D. leguminicola* being pale pink, whereas those of *D. gentneri* are nearly all white except for the thoracic ones, which are pale pink.

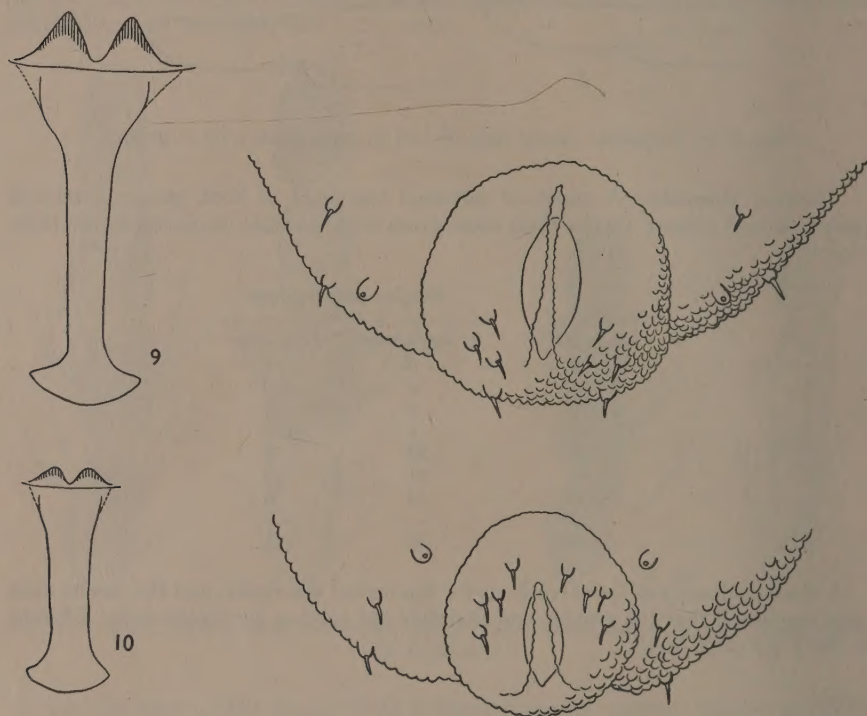
Mature larvae.—The mature larvae are more easily differentiated. Larvae of *D. leguminicola* are a uniform pink, whereas those of *D. gentneri* are yellow, quite often with the anterior fat-bodies bright pink. The lobes of the spatulae differ as described for the previous instar and the overall dimensions of the spatulae are as follows: *D. leguminicola* (fig. 9), stem length 8 times width at middle and 6 times height of lobes; *D. gentneri* (fig. 10), stem length 5 times width at middle and 9 times height of lobes. These figures show that the stem of *D. leguminicola* is longer and narrower than that of *D. gentneri*. In general, the spatula of *D. leguminicola* is larger and more heavily sclerotised than that of *D. gentneri*.

The penultimate (adanal) larval segment in *D. leguminicola* (fig. 9) has the spiracle placed between the setae, whereas in *D. gentneri* (fig. 10) both setae are situated posterior to the spiracles.

(b) Biological differences

Introduction

Thorpe (1956) states that "fixed-action" patterns of behaviour are often as specifically constant as anatomical structures, and are potentially of equal value for phylogenetic studies. In the study of gall midges, Barnes (1953) has shown the practicability of biological experiments in the separation of closely allied species



FIGS. 9-10.—Sternal spatula and anal segment of mature larva of : (9) *D. leguminicola* ; (10) *D. gentneri*.

where separation on morphological characters appears to be impractical. Similarly, Stokes (1954) has used behaviour as the means of identifying two species of *Dasyneura* found on *Viola* species. It is therefore suggested that the behaviour differences described in this section are of no less taxonomic importance than the morphological differences already described.

Material and methods

When this work was commenced a supply of material was available as the late Dr. H. F. Barnes had assembled samples of clover from various counties of England and Wales. In subsequent years further samples were received, mostly from members of the National Agricultural Advisory Service in various parts of the country. In this way it was possible to establish in which counties the various species of midges were present and at the same time to ensure a good supply of material.

Samples, usually of 50 clover heads, were sent to Rothamsted Experimental Station in tightly closed polythene bags. Larvae were placed either in an emergence pot, or in an observation tube, or preserved in 70 per cent. alcohol for later examination. The midges and parasites that subsequently emerged were collected daily, a record being kept of the numbers and sexes of the various species. The observation tubes were constructed of two glass tubes, one of which, having a slightly smaller diameter, was inverted inside the larger tube and the intermediate space filled with white sand. In this way the larvae were given a limited space in which to pupate and observations of "subsoil" activities could be watched at intervals through the glass sides. Once the larvae had been inserted, the tube was corked lightly and enclosed in a cylinder of dark grey paper. By sliding this paper cylinder down, the glass could be exposed and the larvae studied under the microscope at magnifications of up to 400 diameters (Pl. I, fig. 5).

For field observations of biology, small plots of clover (approximately 12×18 feet) were grown, in alternate rows of red and white, from seed or small plants supplied by the National Institute of Agricultural Botany, Cambridge. A number of pots of red and white clover was also maintained in a greenhouse, made reasonably insect-proof by fixing muslin over the windows and kept as nearly as possible at outdoor temperatures. At first the plants were covered with muslin bags supported by bamboo canes, but this was found to hamper the development of the clover, and so a smaller breeding cage, approximately 3 inches high and $1\frac{1}{2}$ inches in diameter, was devised for fitting over individual flower heads. These cages were placed on the clover at a very early stage to ensure that the heads were not midge-infested before the setting up of an experiment. The cage is a cylinder constructed of a celluloid frame covered with fine nylon net, one end being sealed with muslin, and the other extended with a muslin sleeve. It can be slipped over the clover heads and tied to a stake (Pl. I, fig. 1). By using thick string the sleeve can be firmly closed round the stem without damage to the plant. The use of these smaller cages greatly facilitated the watering of the plants and their general maintenance. In order to insert live midges collected from the insectary, a slit was made in the nylon net and this was closed with cotton wool after the midges had been inserted.

Host plant and oviposition

Dasyneura leguminicola always selects green heads of red clover (before the pink of the florets is visible) for oviposition, whereas *D. gentneri* selects fully open florets of white clover (Pl. I, fig. 2), Alsike clover (*T. hybridum*) or red clover. Green heads of white clover growing near red clover were frequently examined and in no instance were eggs found.

Further evidence that *D. leguminicola* will not survive, and probably does not normally oviposit, on white clover, is afforded by the fact that, in several hundred dissections, larvae of this species were never dissected out of the florets of white clover, and no adult was ever bred from white clover samples. Evidence that only green heads of red clover are selected for oviposition by *D. leguminicola* is provided by the fact that in two glasshouse experiments females would not lay on green heads of Alsike clover, in two other experiments females would not lay on open flowers of red clover, and in seven experiments they would not lay on open flowers of white clover. In each case one or more of the experiments were carried a stage further by transferring the females to green heads of red clover, where oviposition immediately commenced, thereby indicating that handling of the midges did not affect their ability to oviposit.

It has been seen that *D. leguminicola* will only oviposit on green heads of red clover (*T. pratense*), but *D. gentneri* has a wider range of host plants. The most important fact, however, is that *D. gentneri* will only oviposit on open florets. In thirteen experiments on green heads of red clover it was never observed to oviposit,

and no midge eggs were found in green heads of white clover in the field. Furthermore, young florets of white clover dissected just before opening were never found to contain eggs or larvae, even at times when large numbers of *D. gentneri* were ovipositing throughout the day for several days.

However, although *D. gentneri* has a preference for open white clover, it will also oviposit on open florets of red clover and Alsike clover. Bishop (1954) on one occasion found eggs on red clover in the position typical for *D. gentneri*, and he suggested that this species might occasionally attack red clover. This possibility was followed up by Barnes (1954), who conducted a preliminary host plant test at Rothamsted Experimental Station. In this experiment *Dasynura* females from white clover oviposited in a previously protected head of red clover, doing so in the open florets. Seventeen days later larvae descended and were placed in an indoor insectary pot from which adults emerged. During the present study these adults were examined morphologically and identified as *D. gentneri*. This was, therefore, the first confirmed case of this species breeding successfully on red clover under experimental conditions.

A number of further experiments of this kind were carried out during the present study, and on eight occasions oviposition was observed on red clover. In four of these experiments adults of the usual *D. gentneri* type were reared. *D. gentneri* oviposits in flowers of red clover under field conditions also: over fifty adults were bred from nine different samples of red clover collected from various parts of England and Wales. Females were also seen ovipositing on open Alsike clover in the field, and larvae of this species were removed from dead heads of this plant.

Not only do the host plant range and stage of growth selected differ in *D. leguminicola* and *D. gentneri*, but also the modes of oviposition. *D. leguminicola* stands on top of, or at the side of, green heads of red clover and thrusts the ovipositor between the florets, laying the eggs singly or, more usually, in batches of up to eleven. The female also frequently extends her ovipositor down the inside of the ligule at the base of the green head and oviposits on the protected florets. *D. gentneri*, on the other hand, selects open florets at a stage just before or just after pollination and, standing astride the keel, inserts the ovipositor between the wing and keel petals (Pl. II, fig. 1), usually depositing only one egg and then moving to the next suitable floret on the head.

Movements of the first instar larvae

The first instar larva of *D. gentneri* travels only a short distance, moving out from between the filaments of the keel and wing petals, negotiating the fused bases of the stamens and thereby reaching the large ovary, which at this stage extends quite far up the florets. Larvae of *D. leguminicola*, however, climb upwards to the base of the calyx lobes on the outside of the floret and then, by moving between petals of the corolla and downwards at the same time, penetrate to the small ovary which is situated at the base of the floret.

Miss Metcalfe (1933a) writes "Entrance into the floret appears to be effected simply by eating a way through. Tiny holes in the calyx and corolla have been found which point to this method of entrance".

This theory seems an unlikely one for several reasons. Firstly, the mouthparts of the first instar larva appear inadequate for the task of cutting through the calyx. Secondly, Creel & Rockwood (1947), Saunders (1882), Folsom (1909) and Comstock (1880) have observed only one method of entry to the ovary, and that is by passing between the petals from the top of the floret down. Thirdly, very careful observations, including the taking of X-ray photographs of infested florets, showed no holes in the calyx and corolla. Miss Metcalfe may have confused the comparatively large holes made by the ovipositors of certain weevils or those made by nectar-seeking humble bees, for the entrance holes of which she writes. The climbing habit

observed in the first instar larva of *D. leguminicola* is thought to be absent in *D. gentneri*. The first instar larva of *D. gentneri* typically situates itself in the indentations in the ovary of white clover, whereas the larvae of *D. leguminicola* usually descend to the base of the red clover ovary to commence feeding.

Symptoms of attack

As *D. leguminicola* commences to feed at an early stage of floret growth, the ovary and corolla are still developing rapidly, and the feeding of the larva and consequent stimulation of the plant cause marked changes to occur. The floret does not extend normally but remains closed so that pollination does not take place, the ovary becomes shrunken and pitted (Pl. I, fig. 4), infested florets are swollen and hardened owing to the galling stimulus of the larva, and a head that has been attacked can easily be detected by the irregular blossoming (Pl. I, fig. 3) and hard compact feel.

When *D. gentneri* commences feeding the ovary is usually fertilized, nourishment of the petals has ceased and the food supply to the ovary is past its peak. For these reasons no obvious galling occurs, the larva feeds from a well-developed, fertilized ovary, and no external signs of attack can be detected on an infested head. Similarly a red clover floret attacked by *D. gentneri* is not stunted or swollen, the general symptoms being as for white clover.

Life cycle

In glasshouse experiments, the time taken from egg to mature larva in *D. leguminicola* averaged four weeks, with a range of 22 to 40 days, whereas in *D. gentneri* it averaged two weeks, with a range of 11 to 16 days. This shows a very marked difference in the time taken for the larva to reach maturity but it must be remembered that, once mature, the larvae of both species remain for long periods in the clover florets, especially during dry weather.

Mature larvae of *D. leguminicola* will normally leave the heads during wet weather and pupate in the soil. This also occurs commonly in *D. gentneri*, but there is a further variation which is not infrequent during dry weather. Mature larvae of *D. gentneri* may spin their cocoons between the petals of dry florets or between the florets themselves (Pl. II, fig. 2), pupate, and emerge as adults from the flower heads. Cocoons were found throughout 1955 and 1956 in white clover and Alsike clover samples collected from various parts of the country. This method of pupating in the heads would appear to be of use in avoiding delay, normally caused by drought, in completing the life cycle, and may influence the number of generations that can be produced each year.

The interval between the entry of the mature larvae into the soil and the emergence of the first adults of *D. leguminicola* varied from 18 to 40 days, with a mean of 27 days in 30 samples set up in the insectary. In *D. gentneri* the range in 70 samples was 10 to 34 days, with a mean of 22 days.

The duration from egg to adult in *D. leguminicola* therefore averaged approximately 8 weeks, whereas in *D. gentneri* it averaged approximately 5 weeks. This means that, whereas *D. leguminicola* can usually complete only two main generations each year in most parts of England, *D. gentneri* may complete four generations annually.

The emergence dates of the overwintering generation of *D. gentneri* differed appreciably from those of *D. leguminicola* in 1955, 1956 and 1957, as shown in the table below. In all these cases the spring emergence of *D. gentneri* was considerably later than that of *D. leguminicola*.

Apparent divergences occurred in various red clover pots set up in the insectary owing to the late emergence of some midges. For this reason all the midges emerging

Year	Dates of Emergence					
	First		Peak		Last	
	<i>D. leguminicola</i>	<i>D. gentneri</i>	<i>D. leguminicola</i>	<i>D. gentneri</i>	<i>D. leguminicola</i>	<i>D. gentneri</i>
1955 .	30th May	12th June	3rd June	30th June	15th July	21st July
1956 .	25th May	19th June	31st May	2nd July	22nd June	29th July
1957 .	3rd May	9th June	14th May	21st June	8th July	5th July

from these pots were later closely examined. It was found that, in all but one case, the pots with late emergences had produced *D. gentneri* adults. It seems therefore that this species, when completing its life cycle on red clover instead of on white, still retains the emergence characters of the species. However, it is interesting to record that on the one occasion when midges emerged very late from red clover and were confirmed as *D. leguminicola*, an experiment was set up with two of the adults in 1955 and the F1 generation was correspondingly late in emerging in 1956. Similarly an experiment, in which males and females of these F1 midges were mated and bred on red clover in 1956, gave rise to a very late F2 spring emergence in 1957. No distinctive morphological characters could be found in any of these midges, but the emergence dates strongly suggest that this was a late-emerging strain.

The difference in time of emergence in *D. gentneri* and *D. leguminicola* is closely paralleled by the time of flowering of white and red clovers. In 1956 the Singlecut and Broad Red clover strains were producing considerable numbers of green heads by 11th June, whereas open flowers of white clover were only present in large numbers approximately two to three weeks later. Similarly, in 1957 green heads of red clover were numerous by 20th May, but open flowers of white clover were not present in any number until the first week in June. It is suggested that these differences in time of flowering must be responsible for the different times of spring emergence of the two species.

Sex ratio of insectary-bred midges

The sex ratio of *D. leguminicola* in 1955 was first generation 34 : 66, second generation 45 : 55, whereas in *D. gentneri* it was 26 : 74 and 33 : 67 respectively. In 1956 the ratio in *D. leguminicola* was first generation 45 : 55, second generation 57 : 43, whereas in *D. gentneri* it was 37 : 63 and 42 : 58 respectively. In each case the number of males increased in proportion to the number of females in the second generation, but in all generations there was a higher proportion of males in *D. leguminicola* than in *D. gentneri*.

Parasites

Three parasites were bred from larvae of *D. leguminicola* in 1956 and 1957, and these were identified as *Tetrastichus inunctus* Nees, *Prosaetogaster demades* Walker, and an *Aphanogmus* sp. (Pl. I, fig. 6). The only parasite bred from *D. gentneri* was identified as *Prosaetogaster error* Fitch.

Distribution

Samples sent in by N.A.A.S. workers in many counties have shown that *D. gentneri* is present on red and white clover in Northumberland, Lincolnshire, Nottinghamshire, Derbyshire, Shropshire, Monmouthshire, Cornwall, Oxfordshire, Hertfordshire, Montgomeryshire, Cardiganshire and Pembrokeshire.

D. gentneri was found to be present on white clover only from Durham, Yorkshire, Cheshire, Leicestershire, Warwickshire, Northamptonshire, Cambridgeshire, Suffolk, Essex, Sussex, Hampshire, Dorset, Devon, Berkshire, Buckinghamshire and Bedfordshire.

Similarly, *D. leguminicola* was bred from red clover samples from Northumberland, Yorkshire, Cheshire, Derbyshire, Nottinghamshire, Lincolnshire, Shropshire, Leicestershire, Warwickshire, Norfolk, Cambridgeshire, Bedfordshire, Hertfordshire, Essex, Buckinghamshire, Oxfordshire, Gloucestershire, Monmouthshire, Berkshire, Kent, Sussex, Hampshire, Devon and Cornwall.

(c) Breeding experiments

From 23 attempted crosses between midges from red and white clover, only four were successful and none of these was confirmed as an interspecific cross. In those cases where the original adults or individuals of the first generation were retained, it was always found that the *Dasyneura* used from red clover was *D. gentneri*, so that an interspecific cross had not occurred. This was also the case in the experiment carried out in 1954 by H. F. Barnes, the female bred from red clover having been mounted during the present study and identified as *D. gentneri*.

The following experiment, carried out in 1957, is typical of the results obtained in many experiments. A male bred from red clover was placed with a female from white clover, and a male from white clover with a female from red clover. After two hours no attempt at mating had been made by either pair, so transfers were made, bringing the white clover midges together and the red clover midges together. Within five minutes both pairs had mated, the mating lasting more than one minute in both cases. A detailed morphological examination confirmed that both specimens from red clover were *D. leguminicola* and that both from white were *D. gentneri*.

It can be seen that the accurate morphological separation of species is of extreme importance in the interpretation of such inter-mating experiments and of many biological observations, especially where the separation of closely allied species is involved.

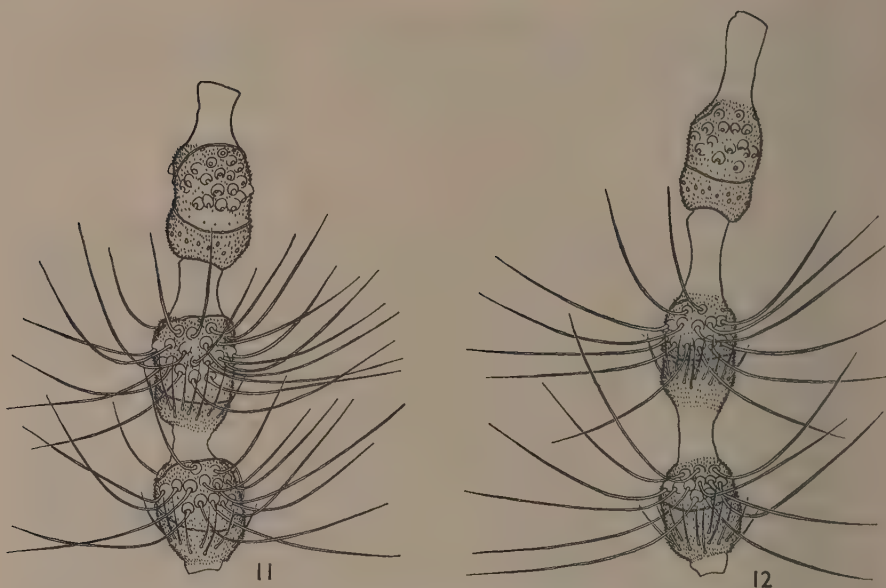
(d) Comparative morphology of other *Dasyneura* species

Having established the importance of using both morphological and biological characters for the separation of *Dasyneura leguminicola* and *D. gentneri*, it was considered advisable to test the usefulness of morphological characters in separating other pairs of closely allied *Dasyneura* species, previously separated mainly on biological grounds. The late Dr. H. F. Barnes suggested that an attempt to separate *D. pyri* Bouché, the Pear Leaf Midge, from *D. mali* Kieffer, the Apple Leaf Midge, would be of value, as experiments and observations had indicated that, while their biologies were distinct, their morphologies were remarkably similar. He also suggested the examination of *D. affinis* Kieffer, the Violet Leaf Midge, and *D. violae* Loew, both of which occur on species of the plant genus *Viola*, but which Miss Stokes (1954) had not succeeded in separating morphologically.

In both pairs very similar differences to those described for *D. leguminicola* and *D. gentneri* were found, but only the most marked distinctions are given here.

Males of *D. mali* and *D. pyri* were found to be easily distinguishable on characters of the third flagellar segment. There are more tubercle-based setae on the ventral surface of this segment in *D. mali* (fig. 11) than in *D. pyri* (fig. 12), and in *D. mali* the microtrichia are distinctly more sparse between the tubercle-based setae and the lower ring of circumfila. The most obvious difference, however, lies in the ratio of stem length to node length. In *D. pyri* the stem is three quarters the length of the node, whereas in *D. mali* it is half the length; this means that the stems in *D. mali* appear much shorter and broader than those of *D. pyri*.

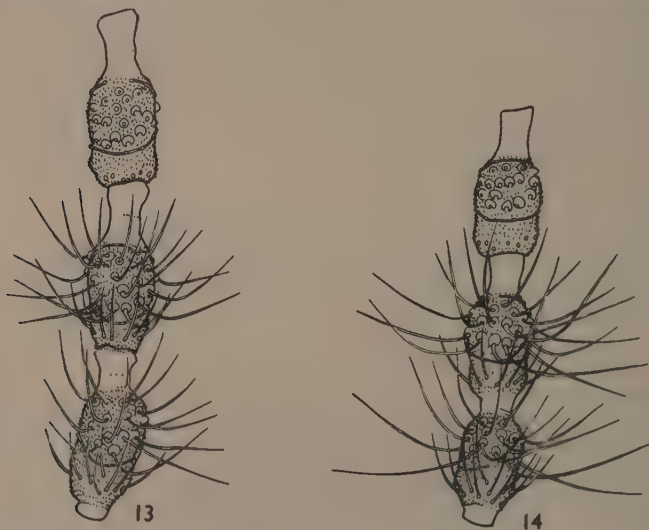
Similarly in *D. affinis* and *D. violae* the flagellar segments of the male antennae show several very marked differences. The fusing of the first and second flagellar segments is incomplete in *D. affinis* (fig. 13), a distinct series of marks and ridges very closely resembling a normal joint between segments being apparent, whereas



FIGS. 11-12.—Ventral view of first three flagellar segments, male, of : (11) *D. mali* ; (12) *D. pyri*.

in *D. violae* (fig. 14) fusion is complete. The third flagellar segment in *D. affinis* is two and three quarter times as long as its width in the middle of the node, whereas in *D. violae* it is only slightly more than twice as long as its width.

It is important, however, when using morphological characters in the separation of closely allied species, to bear in mind that the morphology varies considerably within each species in most Cecidomyiidae, and that it is advisable to examine a number of individuals before drawing conclusions as to their taxonomic position.



FIGS. 13-14—Ventral view of first three flagellar segments, male, of : (13) *D. affinis* ; (14) *D. violae*.

2. *Tricholaba barnesi* sp. n.(a) *Morphology*

Barnes in 1929, 1930 and 1954 bred adults of what were thought to be *Hadrobremia longiventris* Kieffer from red clover samples from various counties in England, and also recorded finding larvae, which he suggested might belong to this genus, from several other samples of red and white clover. A number of these specimens have been mounted during the present study and new material has been bred from numerous other samples between 1955 and 1957 so that a further examination has been possible. The species does not belong to the genus *Hadrobremia*, but is a species of *Tricholaba* Rübsaamen (1917).

In 1909 Kieffer first described a species, *Clinodiplosis trifolii*, from the yellow larvae found in swollen florets of zig-zag clover (*T. medium*). In 1913, however, when he described the adults, he renamed the midge *Hadrobremia longiventris*, having erected a new genus for this species a year previously (1912a).

There are a number of close similarities between his description of *H. longiventris* and the *Tricholaba* species found on clover in England but there are also a number of important differences. Firstly the claws of *Hadrobremia* are all simple, whereas those of *Tricholaba* are toothed on the prothoracic legs and simple on the remainder. The terminal clasp segments of *Hadrobremia* are described by Kieffer as "rather slender, feebly curved", whereas in *Tricholaba* they are rather thick and quite appreciably curved. The lower lamella (ventral plate) in *Hadrobremia* is described as "linear and gradually tapering", whereas in *Tricholaba* it is broad distally, and deeply and broadly emarginate. The larvae also differ slightly: firstly in the anal segments, *Hadrobremia* possessing a more complex anal armature than *Tricholaba*, and, secondly, in the sternal spatula, the lobes of which are described as "separated by a narrow incision" in *Hadrobremia*, whereas they are broadly divided in *Tricholaba*. From these characters it is concluded that the species bred in England definitely does not belong to the genus *Hadrobremia* but is a species of *Tricholaba*. Kieffer's *H. longiventris* therefore remains a distinct species.

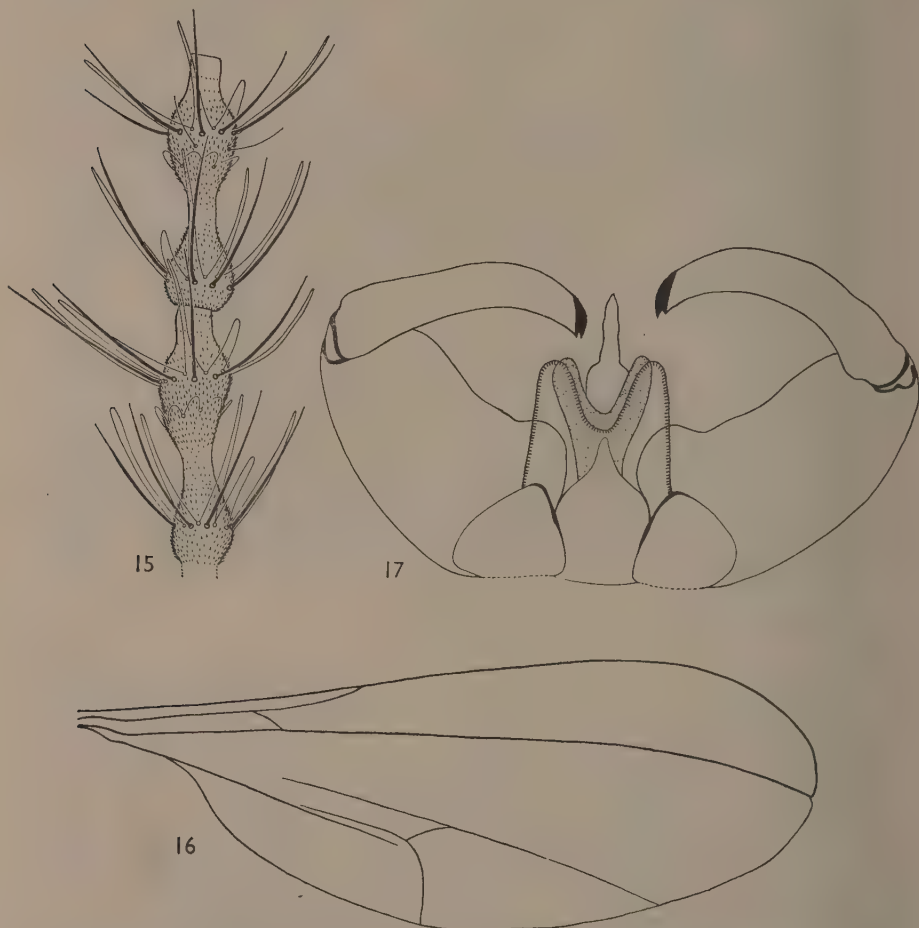
Separation from other Tricholaba species

It was considered possible that it might be the same species as that bred from leaves of clover infested with *Dasyneura trifolii*, namely *Tricholaba trifolii* Rübsaamen. An examination of material of the latter species in the Barnes collection, and of the original description by Rübsaamen (1917), has led to the conclusion that they are distinct species, differing not only in habitat but also in morphology. The node of the third flagellar segment in *T. trifolii* females is three and a half to four times longer than the stem, whereas in the *Tricholaba* species from clover heads it is five times longer. In the males of *T. trifolii* the third flagellar segment is ten and a half to eleven times the diameter of the proximal stems, which are clearly defined, whereas in the *Tricholaba* from clover heads the length of the third flagellar segment is only nine times the diameter of the proximal stem, and the stems run gradually into the nodes. The *Tricholaba* from clover heads also differs from the undescribed species (in the Barnes collection) found with *Dasyneura lupulinae* on *Medicago lupulina*, the length of the third flagellar segment in the male of this undescribed species being eleven times the diameter of the proximal stem. The *Tricholaba* species from clover heads is therefore described as new.

Male.—Length 1.5–2 mm. Antennae grey, 2 + 12; first two flagellar segments fused; third (fig. 15) binodose, with two whorls of hairs and three whorls of circumfila, first and third irregular, second regular and short; stems bearing microtrichia and as heavily pigmented as nodes; total length of segment 8 to 9 times diameter of proximal stem. Face orange, palps four-segmented. Thorax pale orange, legs brownish-grey, tarsal claws toothed on prothoracic legs, simple on other legs. Wings (fig. 16) elongate, third vein uniting with costa slightly before apex of wing. Halteres

orange-yellow. Abdomen slightly darker orange than thorax. Genitalia (fig. 17) yellowish, dorsal plate large, deeply and roundly emarginate; ventral plate roundly emarginate, the lobes diverging; basal clasp segment enlarged basally on inner side; distal clasp segment curved, nearly as long as basal clasp segment and with small setae on outer side of base.

Cotypes (deposited in the Barnes collection): from red clover. Cecid. 12086 and 12011.



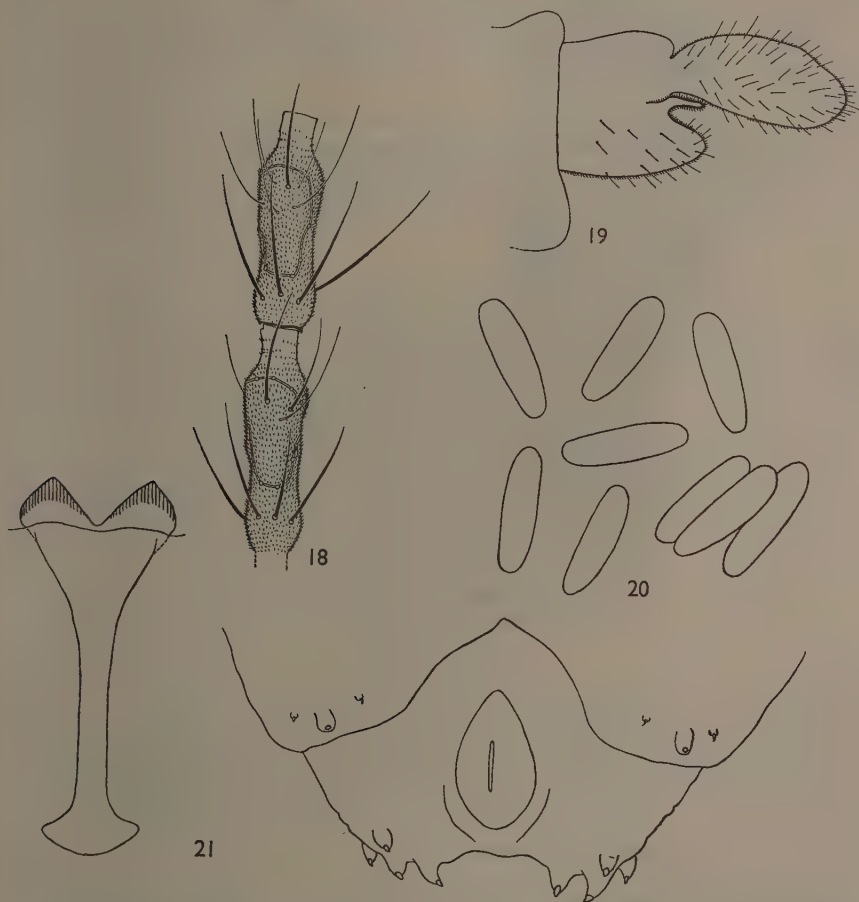
FIGS. 15-17.—*T. barnesi*: (15) male, dorsal view of second and third flagellar segments; (16) wing; (17) male genitalia.

Female.—Length 2-3 mm. Colour as in male except where otherwise stated. Antennae 2 + 12; first two segments fused; third flagellar segment (fig. 18) long, cylindrical, with a short stem possessing microtrichia; node 5 times as long as stem, with two whorls of applied circumfila converging and meeting laterally; two whorls of hairs, one of which is a long basal whorl and the other a further whorl of short and long hairs on distal third of node; length of stem very slightly more than diameter. Abdomen very bright orange; ovipositor (fig. 19) bright yellow, lamelli-form, composed of two slightly elongate, oval lamellae and a small ventral lobe.

Cotypes (deposited in the Barnes collection): from red clover, Cecid. 12029 and 10763; from white clover, Cecid. 12437. 56 additional slides have been deposited in the collection.

Eggs (fig. 20)

Size 0.30 to 0.34 mm. (mean 0.32 mm.) long; 0.08 to 0.10 mm. (mean 0.09 mm.) wide (19 eggs measured). Elongate, rounded at both ends, posterior end very slightly narrower than anterior end. Colour dark orange. Incubation period 3 to 4 days.



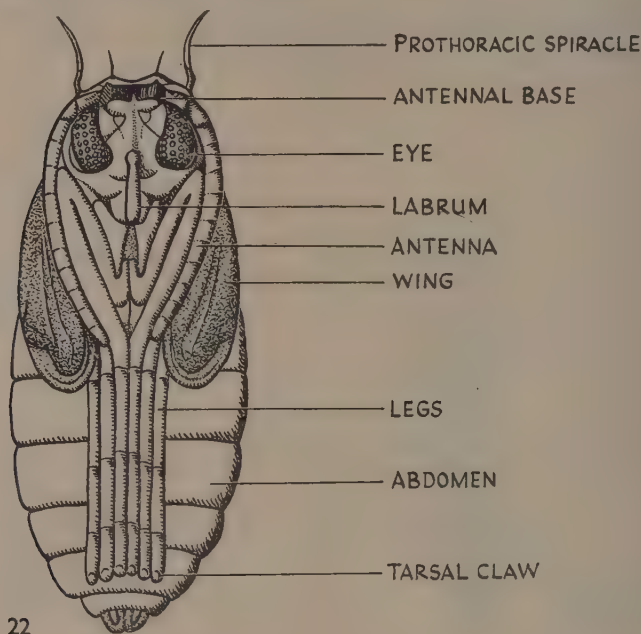
FIGS. 18-21.—*T. barnesi*: (18) female, ventral view of second and third flagellar segments; (19) ovipositor, lateral view; (20) eggs $\times 45$; (21) sternal spatula and anal segment of mature larva.

Fourth instar (mature) larva

Fat-bodies of mature larva yellow and gut contents orange, so that overall colour is yellow ochre. 2.4 to 3 mm. long; antennae two-segmented, second segment being short and digitiform. Eight pairs of abdominal and one pair of thoracic spiracles. Fine "finger-print" sculpturing present on ventral surface of abdominal segments, and minute setae on either side of terminal spiracles. Anus situated ventrally and anal segment bears four pairs of tubercle-based, short, conical setae (fig. 21). Sternal spatula (fig. 21) well-developed with a long, narrow stem and two pointed lobes, broadly divided by a V-shaped emargination.

Pupa (fig. 22)

Approximately 2 mm. long and at first the colour of the mature larva. Leg-sheaths extend to penultimate abdominal segment, and antennal sheaths (in female) extend nearly as far down body as wings. Bases of antennae angular; a small pair of cephalic hairs and two well-developed outward-curving, thoracic spiracle-horns present.



22

FIG. 22.—*T. barnesi*, three-day-old pupa.(b) *Biology**Oviposition*

Oviposition occurs late in the day and most activity was observed in the field between 5 p.m. and 8 p.m. (G.M.T.). In this character *Tricholaba barnesi* differs considerably from the *Dasyneura* species on clover, which oviposit during the sunny part of the day. The female selects green heads of red clover for oviposition, choosing those which have been visible for two or three days, in much the same way as *D. leguminicola*. The translucent orange-red eggs are deposited singly, or in groups of two to four, at the bases of the calyx lobes and the time taken for the deposition of a single egg is approximately 30 seconds. The female stands at the top of the green clover head and curves the ovipositor down between the green florets in the same way as *D. leguminicola*, but it may be distinguished by its larger size and bright orange colour. Females in the field were observed to oviposit on more than one clover head. Adults have been bred from white clover and Alsike clover samples, but oviposition has not been observed on these plants.

It is thought that the newly emerged larvae enter the florets in much the same way as do the larvae of *D. leguminicola*, that is, by climbing between the calyx lobes and making their way between the petals to the base of the ovary. No puncturing of the calyx or corolla could be detected in infested florets.

Florets of red clover infested with *T. barnesi* are stunted in a similar way to that described for *D. leguminicola*, the only difference being that there is less swelling at

the base of the florets and the latter are not quite as stunted (Pl. II, fig. 3). The ovary of an infested floret is small, the style brown and shrunken, yellow-brown pits or marks appear on the surface of the ovary and no seed develops.

The duration of larval development in the florets is 16 to 21 days in early summer, slightly shorter than for *D. leguminicola*. Owing to the failure of the flowers to open and the damage caused by the larva, no fertile seed is produced from an attacked floret of red clover.

Further evidence of the capacity of this species to destroy seed was supplied by an experiment in which a mated *T. barnesi* female oviposited on a green head of red clover which had previously been protected from infestation. A number of larvae developed to maturity, causing stunting of the florets and destruction of the seed. Large numbers of larvae have been found in samples from several counties. *T. barnesi* is therefore considered to be of economic importance to clover seed growers in England.

The mature yellow larvae were observed to leave infested florets of red clover when they were watered in detector cages in the laboratory, thus suggesting that during rain they probably normally leave the heads to pupate in the soil. Mature larvae were observed to possess the jumping habit characteristic of *Contarinia* and certain other genera.

Cocoon-spinning and pupation

Three observation tubes were set up with *T. barnesi* larvae, and the following notes were made during the summer. When entering the soil the larvae use the sternal spatula for clearing obstructions and for making a space for cocoon-spinning, so that the spatula becomes worn. The process of clearing a space for the cocoon takes less than 24 hours and spinning is then commenced. The cocoon is composed of numerous white strands laid down to form a mat-like material which adheres to the surrounding soil particles. The size of the cocoon varies considerably, ranging from 1.2 to 2.6 mm. in length, and it is completed in 3 to 5 days. For a week to 10 days the larva rests or moves about slowly in the cocoon, and then enters the prepupal stage. This is characterised by the inactivity of the larva and the withdrawal of most of the fat-bodies into the posterior two-thirds of the body, leaving the head and thorax transparent, apart from small ocular spots.

After three to five days, pupation takes place, the larval skin splitting dorsally and the pupa escaping from the anterior end. The sternal spatula is shed with the larval skin and can in some cases be seen in the bottom of the cocoon. Approximately ten days after the commencement of pupation the pupa forces its way out of the cocoon with the aid of the sclerotised bases of the antennae and then wriggles to the surface of the soil. When the upper half of the pupa is projecting from the soil, eclosion of the imago takes place.

Life cycle

The intervals between the larvae entering the soil and the first emergence of adults, in eleven experiments, were as follows: 17, 18, 18, 19, 20, 20, 21, 22, 23, 26 and 26 days (mean 21 days). It seems probable that there are at least two generations per annum, as these figures were all obtained from adults emerging at the end of August and early in September from larvae collected at the end of July or early in August. In 1955 the first adult from overwintering larvae emerged on 15th June, and in 1957 the first adult emerged on 19th June. In 1956 adults were found ovipositing in large numbers at the end of July, but mature larvae were found in several samples from 24th July onwards, suggesting that adults had already been present for some time. In 1956 large numbers of larvae were found in red clover up to the end of September.

In general, the generations of *T. barnesi*, particularly the first, are considerably later than those of *Dasyneura leguminicola*, but a certain degree of overlapping occurs and it is not uncommon for both species to be present in a single clover head. On no occasion during the present investigation were both species found in one floret, so that the possibility of this species being dependent on the presence of *D. leguminicola* for its feeding is unlikely. This is the first instance of a definite feeding habit being assigned to a species of the genus *Tricholaba*.

Parasites and predators

The only parasite bred from *T. barnesi* during this study was a small Hymenopteran, *Synopeas* sp., determined by G. E. J. Nixon of the British Museum (Natural History), who states that this genus is under revision. Two larvae of *Lestodiplosis trifolii* introduced into an observation tube with *T. barnesi* were observed to feed on these larvae in the soil. The heads of the *L. trifolii* larvae appeared to be partially inserted into the bodies of the *T. barnesi* larvae, and the surrounding area became white and coagulated; all the larvae attacked in this way died.

Distribution

T. barnesi has been bred from clover samples from the following counties of England and Wales: Yorkshire, Cheshire, Derbyshire, Lincolnshire, Suffolk, Bedfordshire, Hertfordshire, Buckinghamshire, Oxfordshire, Berkshire, Hampshire, Sussex, Denbighshire, Montgomeryshire and Flintshire.

III. PREDATORY SPECIES

1. *Phaenobremia aphidivora* Rübsaamen

(a) *Morphology*

Phaenobremia aphidivora was first described as *Diplosis aphidivora* by Rübsaamen (1891), when he recorded it as feeding on aphids on *Heracleum sphondylium* in Germany.

Nijveldt (1957) has recently made an examination of the *Phaenobremia* material in the Barnes collection and has concluded that there are three types of lower lamella in the male genitalia of this genus. He distinguishes *meridionalis* and *aphidivora* as two distinct cordate types, and *urticariae* as the truncate type. Kieffer (1913), on the other hand, distinguishes only two types, the truncate and the cordate, and with this latter view the writer agrees.

The specimens bred from clover during 1955-57 are certainly of the cordate type and no clear morphological differences can be found between them and the description of *P. aphidivora* by Rübsaamen (1891). These specimens also agree closely with those (in the Barnes collection) bred from *Myzus ornatus* in South Gosford (Barnes, 1929), which Nijveldt (1957) lists as being in the *aphidivora* group. The wing venation agrees closely with the figure of *Diplosis aphidivora* given by Rübsaamen in his original description, where he compares it with the venation of *D. aphidisuga*. The antennal characters also agree with his separation of *D. aphidivora* from *D. aphidimyza*.

Therefore, since Nijveldt (1954, 1955) has also demonstrated that *Phaenobremia aphidivora* has a wide range of prey and an apparently wide geographical distribution, it was considered advisable to group the specimens from clover as *P. aphidivora*.

A description of the species found on clover, with notes on its biology, is given below.

Male.—Length 2 mm. Eyes black. Antennae grey, 2 + 12, stems darker than nodes, third flagellar segment (fig. 23) binodose, basal node globular, distal cylindrical; three whorls of applied circumfila, first irregular, on basal node, second regular, near base of distal node, third irregular, on upper part of distal node; longest circumfila of the irregular whorls on dorsal surface of nodes;

circumfila loops on basal node more than one and a half times as long as the segment ; two irregular whorls of hairs, one on basal node, other near top of distal node ; running parallel with long circumfila loops are long hairs only slightly shorter than circumfila ; stems without microtrichia ; stem of second node (of third flagellar segment) as long as node. Palps ochre, quadriarticulate. Legs ochre, pubescent ; ratio of metatarsus to second segment of tarsus 1 : 13 in prothoracic legs ; tarsal claws toothed on pro- and meso-thoracic legs, simple on hind legs. Wings (fig. 24) pubescent and iridescent ; veins yellowish ; third vein uniting with costa beyond apex of wing ; fifth vein forked ; a small, inconspicuous cross vein unites third vein and subcosta. Abdomen



FIG. 23.—*P. aphidivora* : male, lateral view of second and third flagellar segments.

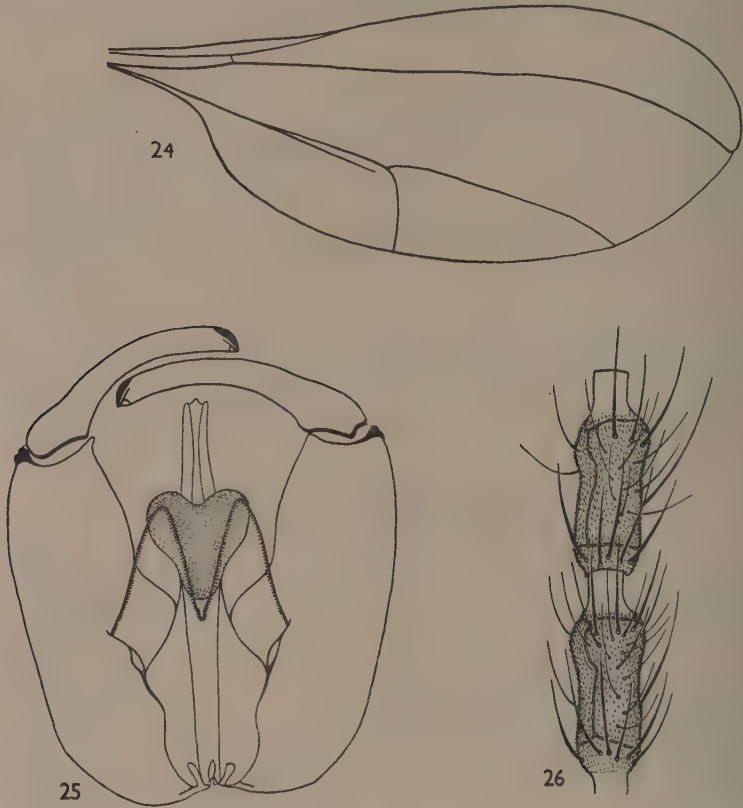
light orange brown. Genitalia (fig. 25) greyish ochre ; dorsal plate consisting of two triangular lobes united at the base ; ventral plate cordate, slightly concave distally ; basal clasp segment long, one and a half times as long as distal clasp segment ; the latter moderately curved.

Female.—As the male, only more pink in colour and slightly longer. Antennae 2 + 12, first two flagellar segments fused, third (fig. 26) cylindrical, nearly 3 times as long as broad ; two whorls of applied circumfila joined by a longitudinal applied series ; two whorls of strong hairs, one distal, the other proximal, and several smaller hairs medially ; neck approximately as long as broad. Ovipositor (fig. 27) ochre in colour, lamelliform with large oval lamellae and an additional broad ventral lobe.

Material bred from red clover infested with *Myzus ornatus* Laing, *Acyrtosiphon pisum* Harris and *Brachycaudus helichrysi* Kltb., in the Rothamsted Lodge greenhouse. Slides have been deposited in the Barnes collection.

Fourth instar (mature) larva

Mature larva pale yellowish-orange with a dark, greenish internal spot. Length 2.5 to 3 mm. Eight pairs of abdominal and one pair of thoracic spiracles present. Sternal spatula (fig. 28) light brown, long and narrow with two lobes. Anal segment (fig. 28) with an elongate anus ventrally and four pairs of terminal, tubercle-based setae, separated by a series of conical projections of the epidermis. Mouthparts not as heavily sclerotised as in *Lestodiplosis trifolii* Barnes.



FIGS. 24-26.—*P. aphidivora*: (24) wing; (25) male genitalia; (26) female, ventral view of second and third flagellar segments.

(b) *Biology*

The eggs of *Phaenobremia aphidivora* are deposited on the hairs on the underside of a leaf or on the stem of a clover plant infested with aphids. The elongate eggs are laid singly and appear to be stuck to the hairs, always resting in an end-on manner. The newly emerged larvae almost immediately commence feeding by inserting their mouthparts between the leg joints, antennal joints or body segments of the aphids, and a third instar larva is capable of draining an aphid half its own size of most of its body fluids within an hour. As an experiment, an immature aphid was placed at one end of a clean leaf and a *P. aphidivora* larva on the other end; after half a minute of apparent searching the larva located the aphid and commenced feeding. This experiment was repeated three times with different individuals with similar results.

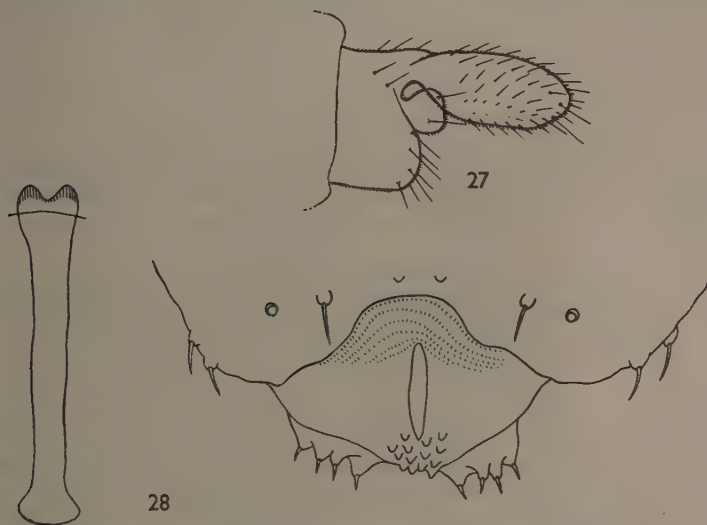
When mature, the larvae crawl or drop to the soil, where they start burrowing.

These larvae have the "jumping" habit characteristic of certain genera and may spring distances of nearly 6 inches. The sternal spatula is used in clearing a space for cocoon-spinning and becomes worn in the process.

During spinning black faeces are passed by the larvae and combined in the construction of the cocoon, which is therefore brownish-black in colour (Pl. II, figs. 4 and 5). This is thought to be the first record of larval faeces in the family Cecidomyiidae. The sternal spatula is shed at pupation.

Distribution

Adults were bred from field samples of red clover from Shropshire and Hertfordshire and also from larvae found feeding on *Myzus ornatus* Laing, *Acyrtosiphon pisum* Harris and *Brachycaudus helichrysi* Kltb., on clover in the greenhouse at



FIGS. 27-28.—*P. aphidivora* : (27) ovipositor, lateral view ; (28) sternal spatula and anal segment of mature larva.

Rothamsted Lodge. Adults emerged at the end of August from a sample collected on 26th July, so that there would appear to be two generations per annum, the second generation larvae overwintering in cocoons in the soil.

Host range of *P. aphidivora*

In June, 1957 the following experiment was carried out with six *Phaenobremia* larvae found feeding on immature *Brachycaudus helichrysi* Kltb. on red clover in the greenhouse. The midge larvae were removed and offered, in succession, different aphid species on various host plants, as follows :

- Macrosiphum rosea* L. on Rose
- Hyperomyzus lactucae* L. on Black Currant
- Myzus cerasi* F. on Cherry
- Microlophium evansi* Theob. on Nettle
- Aphis fabae* Scop. on Bean
- Megoura viciae* Kltb. on Bean

and finally returned to *Brachycaudus helichrysi* Kltb. on red clover. All the larvae fed on all the aphids listed, but a strong preference was shown for the smaller immature forms rather than the later instars. To ensure that feeding was definitely

occurring, the larvae were not moved until one or two aphids had been drained. These midge larvae were placed in an insectary pot on 19th June, 1957, and *P. aphidivora* adults commenced to emerge 13 days later, on 2nd July. The ability of the larvae to survive on a number of hosts demonstrates the probability that this species possesses a wide host range under natural conditions.

It must be made clear, however, that only carefully controlled breeding experiments with midges from various hosts will give a final and reliable answer to the taxonomic relationships of the *Phaenobremia* species from different hosts on various plants.

2. *Lestodiplosis trifolii* Barnes

(a) Morphology

The genus *Lestodiplosis* consists of a large number of midges which are difficult to separate into species morphologically. The male genitalia are all very similar and it is therefore necessary to use other characters for morphological separation. Barnes (1928) found that the proportions of the necks of the flagellar antennal segments were relatively constant and drew up a key to include all the species in his collection at that time. By using this key, it was found that the species bred from red clover during 1955-57 agrees completely with *L. trifolii* Barnes, described on the female only and bred from leaves of clover infested with *Dasyneura trifolii*. As the male has not been described previously a full description is given below.

Male.—Body length one to one and a half mm. Antennae ochre to brown, 2 + 12, first two flagellar segments fused, third (fig. 29) binodose, basal node hemispherical, distal slightly elongate, globular; three whorls of regular circumfila, two whorls of strong setae, more numerous ventrally; a number of small hairs present on ventral surface of nodes; dorsal surface of basal node with very few microtrichia above whorl of circumfila; stem of third flagellar segment over one and a half and not more than three times as long as broad, of tenth four to five and a half times as long as broad and that of twelfth three to five times as long as broad. Face yellow; palps yellow, four-segmented, fourth segment two and a half to three and a half times as long as broad and slightly longer than third. Thorax brownish-black dorsally, pale yellow-orange laterally. Legs yellowish-grey, darker at joints of tarsal segments; tarsal claws simple, about as long as empodium. Wings pubescent, with faintly pigmented areas which bear greater numbers of scales, giving appearance of dark spots; third vein uniting with costa at apex of wing (fig. 30); fifth vein forked. Halteres pale yellow. Abdomen bright orange-red with a dark interior spot anteriorly. Genitalia (fig. 31) greyish-yellow; basal clasp segment with large triangular basal lobe on inner side; dorsal plate deeply emarginate, the lobes broadly rounded; ventral plate as long as dorsal plate, broadly rounded apically; natural positions of dorsal and ventral plates nearly at right angles to basal clasp segment.

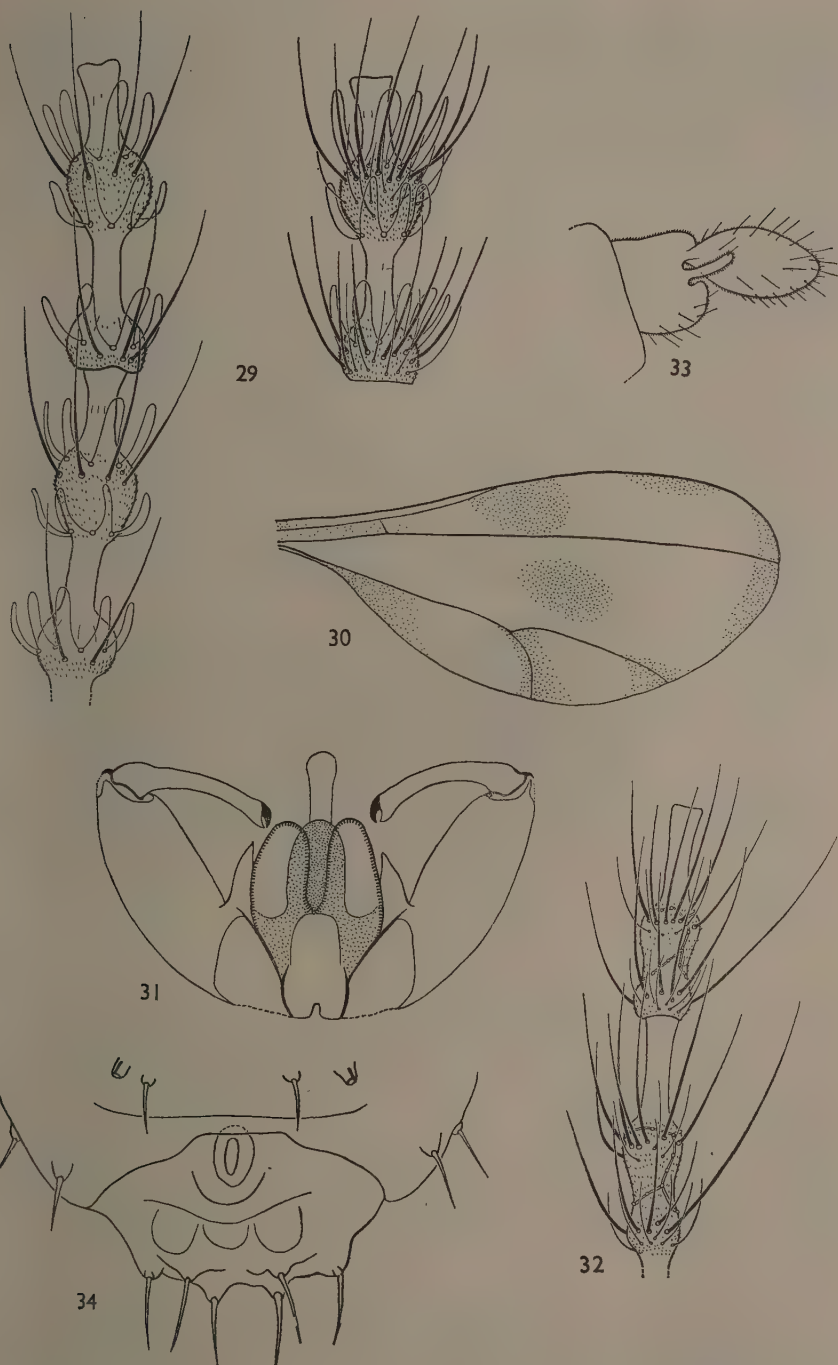
Cotype (slides deposited in the Barnes collection): from white clover, Cecid. 12221 and 12229; from red clover, Cecid. 12237 and 12241. In addition a further 25 slides have been deposited in the Barnes collection. *Female type*: Cecid. 338, bred by H. F. Barnes from *Dasyneura trifolii* F. Loew on white clover from Kent.

Mature larva

2.5 to 3 mm. long and varying in colour from pinkish-orange to deep red-orange, with many intermediate shades. Pronounced pseudopods present ventrally, a feature typical of *Lestodiplosis* larvae, and a dorsal anus, a peculiarity of this genus. Three pairs of anal setae, 2 pairs terminally and one pair dorsally (fig. 34). Eight pairs of abdominal and one pair of thoracic spiracles; terminal abdominal spiracles are dorsal whereas all other pairs are lateral. No sternal spatula but mouthparts very strongly developed and sclerotised (Pl. II, fig. 6). Antennae two-segmented the second segment long, thin and tapering, about as long as distance between bases of antennae.

(b) Biology and distribution

Larvae of *Lestodiplosis trifolii* were frequently found in samples of red clover and white clover from several counties in England and Wales. Specimens were



FIGS. 29-34.—*L. trifolii*: (29) male: (left) dorsal view of second and third flagellar segments, (right) ventral view of third flagellar segment; (30) wing showing darker areas; (31) male genitalia; (32) female, ventral view of second and third flagellar segments; (33) ovipositor, lateral view; (34) anal segment of mature larva.

reared from both red and white clover from Lincolnshire, Nottinghamshire, Norfolk, Shropshire, Derbyshire, Hertfordshire, Buckinghamshire, Oxfordshire, Devonshire, Cornwall, Kent and Flintshire. In addition they were bred from white clover samples from Co. Durham, Bedfordshire, Dorset, Montgomeryshire and Cardiganshire; and from red clover from Cardiganshire and Sussex.

L. trifolii larvae were always found when larvae of other genera were present and it seems most likely that they feed on such larvae from the early stages onwards. Barnes (1928) records *Dasyneura trifolii* on white clover leaves as the host. It seems probable that they also feed on *D. leguminicola*, *D. gentneri* and *T. barnesi* in the flower heads. In observation-tube experiments containing several genera, *L. trifolii* larvae were observed to prey on *D. leguminicola* and *T. barnesi* in the soil, selecting those specimens which had not yet commenced cocon-spinning.

L. trifolii does not spin a cocoon in the soil but pupates naked. On a number of occasions larvae were observed to pupate in the flower heads. Mature larvae were never found on young heads of clover, and so it is presumed that oviposition occurs on mature heads, the female probably seeking florets inhabited by other midge larvae.

In 55 samples of red and white clover, the period from collection of the sample to emergence of the first adult in the Rothamsted insectary varied from 6 to 27 days, with a mean of 17 days. In 1955 the spring emergence commenced on 26th May (apart from one emergence on 9th May) reaching a peak on 29th June. In 1956 numbers were generally lower but emergence commenced on 25th May and reached a peak on 7th July. In 1957 emergence commenced on 12th June and reached a peak on 18th and 19th June.

Owing to the relatively small numbers bred it is not known how closely these dates agree with field emergences. There are certainly two generations each year, and, in view of the short pupal period, probably more. The latest insectary emergence in 1954 was 3rd October; in 1955, 22nd September; and in 1956, 16th September.

The sex ratio of adults emerging in the insectary was always predominantly female. The ratio in 1954 (second generation only) was 24 : 76, in 1955, 32 : 68 and in 1956, 34 : 66. Quite large numbers were bred from some clover samples and it seems probable that these midges could have a marked effect in reducing numbers of *Dasyneura* and *Tricholaba* and may therefore be of economic importance.

IV. OTHER SPECIES AND GENERA

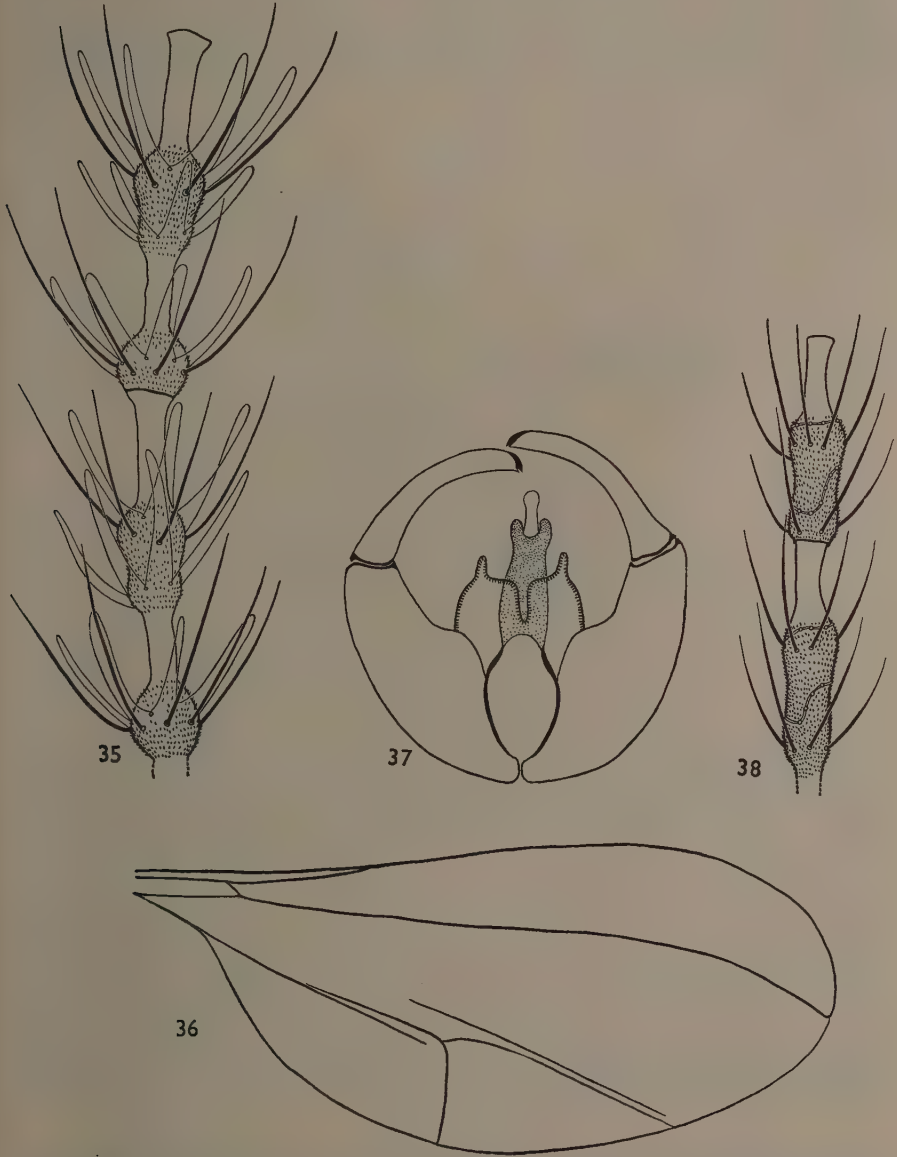
1. *Clinodiplosis leguminicola* sp. n.

(a) *Morphology*

Species of the genus *Clinodiplosis* are found on a very wide range of host plants, but are morphologically very similar. However, an examination of the literature of described species and a careful inspection of the slide material in the Barnes collection have shown that differentiation can be achieved on characters of male genitalia (especially the dorsal plate), the proportions of the third flagellar segment, wing venation, and the sternal spatula of the larva. Colour and size may sometimes be of value, but variation within a species makes this less reliable. As the specimens bred from red and white clover during this study are considered morphologically similar and do not agree with any of the described species (or the undescribed species in the Barnes collection) it was considered advisable to describe a new species.

Male.—Body length 2 mm. Eyes black. Antennae 2 + 12, first two segments almost colourless; third flagellar segment (fig. 35) binodose, basal node subglobular, distal one and a half times as long as its broadest point, very slightly constricted medially; distal neck 4 times as long as its diameter; three whorls of regular circumfila, third whorl nearly as long as neck; two whorls of hairs longer than necks. Mouthparts yellowish-grey; palps grey, quadriarticulate. Thorax pale ochre, halteres long and pale yellow. Wings (fig. 36): third vein uniting with costa beyond apex of wing; fifth vein forked. (In several specimens the fold just anterior to first

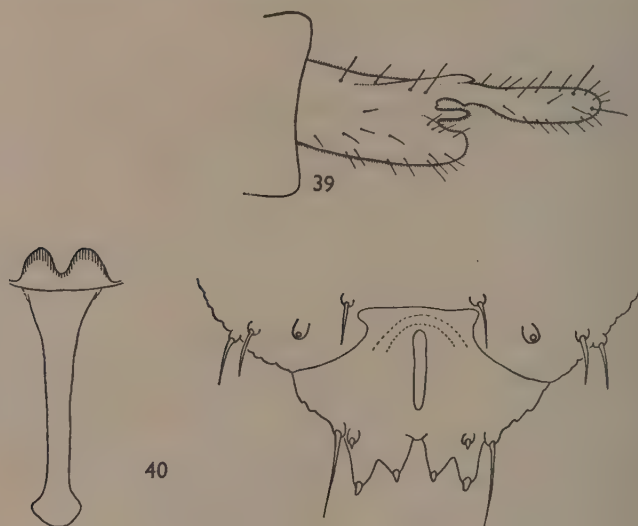
fork of fifth vein possesses a vestigial vein). Tarsal claws curved nearly at right angles, toothed on fore-legs only. Abdomen light ochre, each segment bearing two light brown bands from which long setae arise. Genitalia (fig. 37) pale ochre, pubescent; ventral plate longer than basal clasp segment, narrower, elongated distally, and roundly emarginate apically; dorsal plate deeply cleft, lobes extending to smaller lobes apically on outer side only; basal clasp segment enlarged basally on inner side; distal clasp segment moderately slender and slightly curved.



FIGS. 35-38.—*C. leguminicola*: (35) male, ventral view of second and third flagellar segments; (36) wing; (37) male genitalia; (38) female, ventral view of second and third flagellar segments.

Cotypes (deposited in the Barnes collection): from white clover, Cecid. 12180; from red clover, Cecid. 12044. A further 33 slides have been deposited in the collection.

Female.—Body length 2 mm. Colour as in male but slightly darker. Antennae 2 + 12; third flagellar segment (fig. 38) nine to ten times as long as diameter of stem; stem well defined; two whorls of applied circumfila united laterally by a short longitudinal series; two whorls of hairs and sometimes a single tubercle-based hair laterally. Thorax rust yellow, legs pale orange-yellow; wing as in male. Abdomen pale orange-brown with broad, dark tergites and sternites. Ovipositor (fig. 39) pale yellow, lamelliform, composed of two oval lamellae and a small ventral lobe.



FIGS. 39–40.—*C. leguminicola*: (39) ovipositor, lateral view; (40) sternal spatula and anal segment of mature larva.

Cotypes (deposited in the Barnes collection): from white clover, Cecid. 12183; from red clover, Cecid. 12049. A further 34 slides have been deposited in the collection. A number of slides (males: Cecid. 4569–4573; females: Cecid. 4579–4580; larvae: Cecid. 1762) of a *Clinodiplosis* from red clover seed dust, already in the collection, are considered to belong to the same species.

Larva

Mature larva 2 to 2.5 mm. long and varying in colour from almost white to pale orange. Weakly developed ventral pads present and probably assist in locomotion. Anal segment (fig. 40) includes a ventral anus, a pair of small, ventral, tubercle-based short setae, a pair of long terminal setae, and two pairs of well-developed terminal tubercles with squat, rounded, apical setae. Eight pairs of abdominal and one pair of thoracic spiracles; the first seven abdominal spiracles are lateral, the terminal pair dorsal. Sternal spatula (fig. 40) well-developed, bilobed, with a stem 8 to 9 times as long as its width in the middle. Second segment of the palps moderately long, not tapering but rounded apically.

(b) *Biology and distribution*

Barnes first bred adults of *C. leguminicola* from six samples of white clover and one of red clover in 1954, and suggested that they were inquilines. This has been supported by observations during 1955 and 1956. Larvae were found in mature

heads of red and white clover, usually within the florets and commonly together with a larva belonging to some other genus. In the latter case the other larvae showed no signs of injury, and when alone in a floret there were no signs of damage to the ovary or other flower parts. The exact feeding habits of *C. leguminicola* therefore remain doubtful.

In detector cages the larvae left the heads and dropped into the water below, and it is therefore probable that they pupate in the soil.

In 1954 (Barnes, *unpublished data*) the second generation emergence took place from 30th August to 13th September in six white clover samples. In 1955, spring emergence from samples of red and white clover took place from 30th May to 25th September, the greatest numbers emerging during June; emergence was very sporadic after 25th July. The second generation emerged from 18th July to 28th September. In 1956 emergence commenced on 31st May, ending on 19th August, with the peak during June and only very scattered emergences after the first week in July. The second generation (from samples collected in the field early in the summer) commenced emergence on 27th July, ending, rather early, on 31st August. In 1957 spring emergence commenced on 23rd May, slightly earlier than in previous years, as might have been expected from the mild winter and warm spring.

C. leguminicola adults were bred from samples from eleven counties of England and Wales: Lincolnshire, Nottinghamshire, Shropshire, Hertfordshire, Buckinghamshire, Berkshire, Kent, Devonshire, Dorset, Flintshire, and Cardiganshire. In addition Barnes (*unpublished data*) recognised larvae in samples from Cornwall and Oxfordshire.

2. *Giardomyia britannica* sp. n.

(a) *Morphology*

The genus *Giardomyia* was erected by Felt (1908) for certain species presenting marked differences from *Octodiplosis* Giard, and up to the present date contains only seven described species, all from America, namely: *photophila* (type species), *menthae*, *noveboracensis*, *emarginata*, *hudsonica*, *montana* (Felt, 1920) and *rhododendri* (Felt, 1939), all described by Felt. No female has previously been described for this genus and the rearing of midges of both sexes from several samples of clover during the present study comprises the first record of this genus in Britain.

Male.—Body length 1 mm. Colour pale yellow. Palps quadriarticulate. Antennae 2 + 12, nearly twice as long as body; first and second flagellar segments fused; third (fig. 41) binodose, basal node subglobular, distal nearly as wide as long; three whorls of well-developed circumfila, two whorls on second node; two whorls of hairs slightly longer than stems; stems 3 and 4 times longer than their diameters. Tarsal claws bent nearly at right angles, simple on all legs. Prothoracic legs: ratio of metatarsus to second tarsal segment 1:6. Wings pubescent; third vein uniting with costa beyond apex of wing (fig. 42); fifth vein forked. Genitalia (fig. 43): basal clasp segment not lobed; distal clasp segment curved, nearly as long as basal clasp segment; dorsal plate short, rounded and triangularly emarginate; ventral plate longer than basal clasp segment, slender, deeply and roundly emarginate apically.

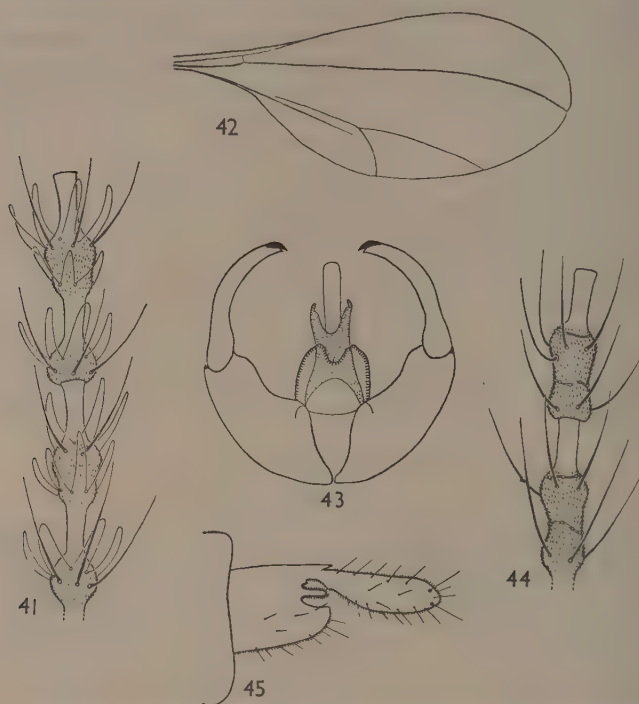
Cotypes (deposited in the Barnes collection): from red clover, Cecid. 12001 and 10765 (all three specimens on slide).

Female.—Body length 1 mm. Colour, pale yellow. Antennae 2 + 12, first two flagellar segments fused, third (fig. 44) cylindrical, node length 3 times its diameter; stem 3 times longer than its diameter; two whorls of applied circumfila, two whorls of hairs, and a single intermediate hair with tubercle base situated ventrally. Ovipositor (fig. 45) lamelliform, lamellae 4 times as long as broad, with a small additional lobe situated ventrally.

Cotypes (deposited in the Barnes collection): from red clover, Cecid. 10766 (all three specimens on slide) and 10767 (all four specimens on slide); from white clover, Cecid. 12099.

These midges were first thought to belong to the genus *Clinodiplosis*, but the absence of teeth on the claws later indicated that this was not the case. It can be distinguished from the other species of the genus *Giardomyia* by the colour, male genitalia, and the proportions of the fifth antennal segment of the male.

The larva is unknown, but in view of the size and colour of the adult may be expected to be small, and pale yellow in colour.



FIGS. 41-45.—*G. britannica*: (41) male, dorsal view of second and third flagellar segments; (42) wing; (43) male genitalia; (44) female, ventral view of second and third flagellar segments; (45) ovipositor, lateral view.

(b) *Biology and distribution*

Little is known of the biology of this genus. Felt (1920) bred one species from a stem gall of mint but all others were captured. It is possible that the larvae of *G. britannica* may cause galls on the heads of clover but it seems unlikely that the species is of any economic importance.

Adults have been bred from samples of white clover from Kent and Shropshire, and from samples of red clover from Shropshire, Montgomeryshire and Cardiganshire.

There appears to be only one generation each year in Harpenden. Emergence commenced in mid-June in 1955 and 1956, and ended early in August.

3. *Isodiplosis deuthera* sp. n.

(a) *Morphology*

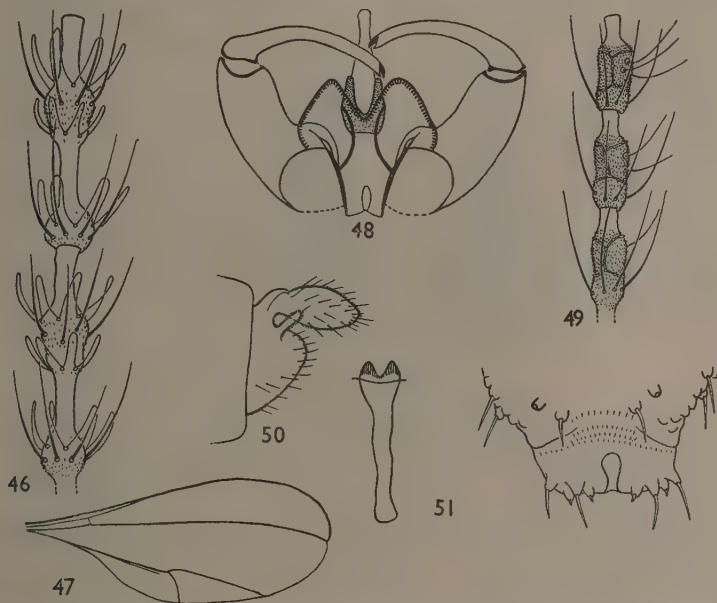
The genus *Isodiplosis* was erected for a single species by Rübsaamen in Germany and he states (Rübsaamen, 1912):

"It is easy to distinguish between the *Isodiplosis* and the similar *Lestodiplosis* females by the shorter stems of the antennal segments of the former, the closely

applied circumfila and the absence of the black spot in the first segment of the abdomen, which in the case of *Lestodiplosis* and its relations is only seldom missing."

Both larvae and adults of a midge belonging to this genus were found during 1955 and 1956, and from this material a new species is now described.

Male.—Body length 1 mm. Colour very pale orange. Antennae $2 + 12$, first two flagellar segments fused; third (fig. 46) binodose, basal node subglobular, distal longer and slightly constricted medially; three rows of circumfila, two on distal node, one on basal; one whorl of regular hairs on each node; very few microtrichia on dorsal surface of basal node. Palps quadri-articulate. Wings pubescent; third vein uniting with costa slightly beyond apex of wing (fig. 47), fifth vein forked. Tarsal claws all simple, pulvilli very small. Genitalia (fig. 48): basal clasp segment with obtuse lobe situated basally on inner side; distal clasp segment curved, slender; dorsal plate broadly, triangularly emarginate; ventral plate fairly narrow, as long as dorsal plate, deeply emarginate, the lobes parallel; style extending well beyond ventral plate.



FIGS. 46-51.—*I. deutera*: (46) male, dorsal view of second and third flagellar segments; (47) wing; (48) male genitalia; (49) female, lateral view of third and fourth flagellar segments; (50) ovipositor, lateral view; (51) sternal spatula and anal segment of mature larva.

Cotypes (deposited in the Barnes collection): from Alsike clover, Cecid. 12030 and 12091: from red clover, Cecid. 12095.

Female.—Slightly more than 1 mm. long, very pale pinkish-orange. Antennae $2 + 12$, first two flagellar segments fused; third (fig. 49) cylindrical with distinct neck, neck one and a half times as long as its diameter; two whorls of applied circumfila joined laterally by a longitudinal series, a single basal whorl of hairs, and two or three tubercle-based hairs situated half way up node on ventral side; node of terminal (twelfth) flagellar segment one and a half times as long as distal extension. Ovipositor (fig. 50) short, lamelliform, composed of two oval lamellae and a small ventral lobe.

Cotypes (deposited in the Barnes collection): from Alsike clover, Cecid. 12092 and 12093. Larval slides 12414 and 12415 from Alsike clover also deposited in the Barnes collection.

Mature larva

Length 1 to 1.4 mm. Colour, pale pinkish-orange. Anal segment (fig. 51) with two pairs of tubercle-based long setae and two pairs of tubercle-based short setae. Eight pairs of abdominal and one pair of thoracic spiracles. Sternal spatula (fig. 51) with two clearly defined lobes, but the stem somewhat wavy and not very strongly sclerotised.

(b) *Biology*

Rübsaamen (1912) described the feeding habits of the only other described species of this genus, *I. involuta*, as follows :

"The reddish-grey larvae live in the cigar-like leaf rolls produced by *Rhynchites betuleti* on *Vitis vinifera*, together with the larvae of that beetle and those of *Clinodiplosis rhynchiton*. They probably live on the decomposition products produced in those leaf rolls or on spores of a fungus, and it is evident that the larvae do not swallow the spores completely, but only empty them by sucking. It is therefore useless to search in the gut of such larvae for whole spores as has been done by other investigators."

I. deutera may also feed on decomposition products or fungus spores, since numerous larvae were found in a sample of dead and decayed heads of Alsike clover collected in the field.

Six of these larva from Alsike clover were placed in an insectary pot on 30th July and four adults emerged on 5th August. The period spent in the soil, including the pupal period, was therefore 16 days. From this evidence it is concluded that there are at least two generations, and possibly more, each year.

Material has been bred from flower heads of red clover from Lincolnshire, and from white clover and Alsike clover from Hertfordshire.

4. *Brachyneura squamigera* Winnertz

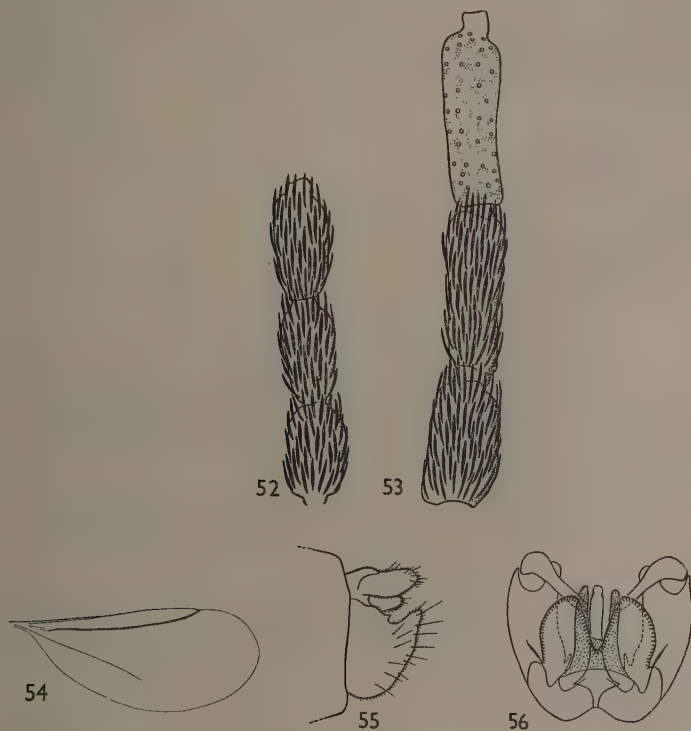
The *Brachyneura* specimens in the Barnes collection have been examined (these are specimens bred from white clover and wheat ears), and those bred from white clover flowers were found to agree closely in morphology with descriptions of *B. squamigera*.

Winnertz (1853), in his original description, described and figured *B. squamigera* as having the body and wings almost entirely covered with scales; palps four-segmented; antennae elongate, 13-segmented in both sexes, the flagellar segments cylindrical. Edwards (1937), however, mounted and examined the types of *B. squamigera*, as well as additional fresh material of the species, and amended Winnertz's description as follows :

"The antennae in both sexes have 2 + 10 (not 13) segments, the indication of a minute, terminal segment being an illusory appearance in the dry specimen only; the flagellar segments are gradually lengthened and not shortened (*i.e.* the distal segments are somewhat longer instead of much shorter than the proximal ones, as incorrectly shown in Winnertz's figures); the palps have 3 segments (not 4). Circumfila are present in the male (though difficult to trace except in a denuded and stained mount) in the form of sinuous threads running the whole length of the segment; they have transverse connections at each end of the segment, but few or none intermediately. Only an apical circumfilum in the female antenna can be made out in the specimens examined. On the front legs the claws are rather large and bifid; on the middle and hind legs they are rather smaller, simple, and very pale (often very difficult to distinguish unless the scales have been removed). The empodium is not easy to make out; it is more evident and apparently longer in some specimens than in others, in the type male about half as long as the claws. Hypopygium of male and tenth tergite (upper lamella) divided into two rounded lobes, tenth sternite (lower lamella) deeply divided into two linear lobes; coxite densely scaly like rest of body and legs. Ovipositor short, lamellate, not extensile".

To Edwards' description the following can now be added :

Third flagellar segment (fig. 52) of female antennae twice as long as broad, the scales slightly shorter and broader than in the male. Third flagellar segment of male with the bases of the scales arranged in groups (fig. 53). Wings (fig. 54) : third vein well-developed, uniting with costa well before apex of wing ; fifth vein simple ; surface covered with many small curved scales. Halteres covered with small, broad, striated scales. Ovipositor (fig. 55) lamelliform, composed of two lamellae and an additional ventral lobe two thirds as long as the lamellae.



FIGS. 52-56.—*B. squamigera* : (52) female, ventral view of first three flagellar segments ; (53) male, ventral view of first three segments (scales removed from third segment to show insertions of circumfila (circles) and points of insertion of scales (dots)) ; (54) wing denuded of scales ; (55) ovipositor, lateral view ; (56) male genitalia.

The male genitalia (fig. 56) agree closely with those figured by Edwards (1937) from Winnertz's type male.

Adults were bred by Barnes in 1954 from samples of white clover from Oxfordshire, Shropshire and Cardiganshire, emergence taking place from 1st to 30th September from samples collected between 9th and 15th July. No specimens were bred from samples during 1955-57. The mode of life is unknown.

5. *Contarinia* sp.

In 1954 Barnes (1954) found what were thought to be *Contarinia* larvae in a sample of red clover from Sussex. He based his primary diagnosis on their yellow colour, the shape of the anal segment, and the "jumping" habit. After mounting, the author has confirmed that they are *Contarinia* larvae. It is not considered

advisable to erect a new species on the basis of larval characters only. A description is given but no specific name is assigned. Although these larvae appeared only in a single sample, the possibility of their being of potential economic importance remains unaltered.

Larva.—2 mm. long, yellow in colour. Eight pairs of abdominal and one pair of thoracic spiracles present. Anal segment (fig. 57) with 3 pairs of tubercle-based short setae, one pair being very small, and the setae of the largest pair short and conical; anus situated ventrally. Sternal spatula (fig. 57) well-developed, with two clearly-defined, moderately pointed lobes. Mouthparts not very strongly sclerotised; antennae short and 2-segmented.

Specimens (in the Barnes collection): from red clover, Cecid. 12434 (all four specimens on slide).

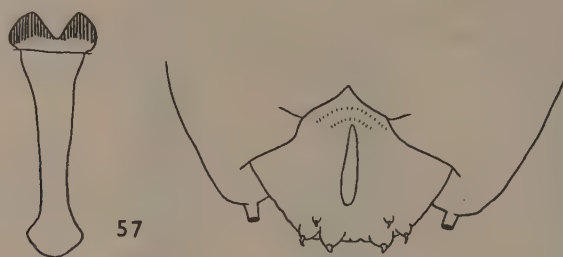


FIG. 57.—*Contarinia* sp., sternal spatula and anal segment of larva from red clover flower heads.

V. KEY³ TO MALES, FEMALES AND LARVAE OF GALL MIDGES INHABITING CLOVER FLOWER HEADS

Males

- | | | |
|---|--|--|
| | Third flagellar segment of antennae single-noded, with applied circumfila . . . | 1 |
| | Third flagellar segment of antennae binodose, with looped circumfila . . . | 3 |
| 1 | Antennae thickly covered with dark brown scales (fig. 53), fifth vein simple, not forked (fig. 54) . . . | <i>Brachyneura squamigera</i> Winnertz |
| | Antennae without scales, fifth vein forked . . . | 2 |
| 2 | Genitalia with projection at base of basal clasp segments which is nearly as broad as ventral plate, lightly sclerotised and regular (fig. 1); rudimentary vein, proceeding towards third vein from point where first radial vein and costa fuse, sometimes present (fig. 4). (<i>Occurs on red clover, T. pratense, only</i>) . . . | <i>Dasyneura leguminicola</i> Lintner |
| | Genitalia with roots of basal clasp segments united to form a slender, strongly sclerotised rod, bordered by an irregular, lightly sclerotised projection (fig. 2); rudimentary vein (as above) never present. (<i>Occurs mainly on white clover, T. repens, but also occurs on red clover, T. pratense, and Alsike clover, T. hybridum</i>) . . . | <i>Dasyneura gentneri</i> Pritchard |
| 3 | Wings pubescent with localised pigmented areas which bear a greater number of small scales, giving appearance of dark spots (fig. 30); natural position of dorsal and ventral plates of genitalia nearly at right angles to basal clasp segment (fig. 31); abdomen bright orange red with a dark internal spot anteriorly situated . . . | <i>Lestodiplosis trifolii</i> Barnes |
| | Wings without dark spots . . . | 4 |
| 4 | Dorsal circumfila of first and third whorls of third flagellar segment greatly extended, nearly twice as long as segment (fig. 23); genitalia: ventral | |

³ Keys based mainly on generic characters.

plate cordate, slightly concave distally, basal clasp segment one and a half times as long as distal clasp segment (fig. 25)

Phaenobremia aphidivora Rübsaamen

Circumfila loops never longer than length of third flagellar segment 5

- 5 Stems of third flagellar segment pigmented, bearing microtrichia (fig. 15); genitalia: dorsal plate deeply emarginate, ventral plate roundly emarginate with diverging lobes, basal clasp segment enlarged basally on inner side (fig. 17) **Tricholaba barnesi** sp. n.

Stems of third flagellar segment without microtrichia and not pigmented 6

- 6 Ventral plate of genitalia one to one and a half times as long as its width in the middle, deeply emarginate, not longer than dorsal plate; dorsal plate broadly, triangularly emarginate (fig. 48) **Isodiplosis deutera** sp. n.

Ventral plate two to three times as long as its width in the middle, longer than dorsal plate 7

- 7 Body length 1 mm. Tarsal claws simple on all legs; genitalia: dorsal plate short, rounded and triangularly emarginate (fig. 43)

Giardomyia britannica sp. n.

Body length 2 mm. Tarsal claws toothed on fore legs only; genitalia: dorsal plate narrowly, deeply cleft, the lobes extending to shorter lobes on outer sides only (fig. 37) **Clinodiplosis leguminicola** sp. n.

Females

Third flagellar segment of antennae without distinct stem 1

Third flagellar segment with distinct stem 3

- 1 Antennae (fig. 52) and wings thickly covered with dark brown scales; ovipositor (fig. 55) lamelliform, composed of two lamellae and an additional ventral lobe two-thirds as long as the lamella; fifth wing vein not forked (fig. 54) **Brachyneura squamigera** Winnertz

Antennae not thickly covered with scales; ovipositor extensile, not lamelliform; fifth vein forked 2

- 2 Third flagellar segment (fig. 5) nearly twice as long as broad medially, with 7 to 16 tubercle-based setae; most tubercle-based setae situated ventrally but always some situated dorsally; ratio of first to second tarsal segment from 6:1 to 9:1; terminal lobe of ovipositor (fig. 7) as broad as remainder of ovipositor when viewed laterally. (*Occurs on red clover only.*)

Dasyneura leguminicola Lintner

Third flagellar segment (fig. 6) one and a half times as long as broad medially, with 4 to 10 tubercle-based setae; seldom more than one tubercle-based seta situated dorsally; ratio of first to second tarsal segment from 4:1 to 6:1; terminal lobe of ovipositor (fig. 8) two-thirds as broad as remainder of ovipositor when viewed laterally. (*Occurs on red clover, white clover and Alsike clover*) **Dasyneura gentneri** Pritchard

- 3 Wings pubescent with localised pigmented areas which bear greater numbers of small scales giving appearance of dark spots (fig. 30); third flagellar segment of antennae (fig. 32) five and a half to six and a half times as long as breadth of node; ovipositor (fig. 33) lamelliform

Lestodiplosis trifolii Barnes

Wings without dark spots 4

- 4 Stem of third flagellar segment pigmented, bearing microtrichia (fig. 18); ovipositor (fig. 19) lamelliform, composed of two large, slightly elongate oval lamellae and a small ventral lobe; abdomen very bright orange-red

Tricholaba barnesi sp. n.

Stem of third flagellar segment without microtrichia and not pigmented 5

- 5 Neck of third flagellar segment one to one and a half times as long as broad 6

Neck of third flagellar segment two and a half to three times as long as broad 7

- 6 Length 2 mm.; neck of third flagellar segment (fig. 26) approximately as long as broad, node bearing a single basal whorl of hairs and two to three tubercle-based hairs situated medially on the ventral side; ovipositor

- (fig. 27) lamelliform with large oval lamellae and an additional broad ventral lobe **Phaenobremia aphidivora** Rübsaamen
- Length 1 mm.; neck of third flagellar segment (fig. 49) one and a half times as long as broad, bearing two whorls of strong hairs, one distally, the other proximally and also several finer hairs medially; ovipositor (fig. 50) short, composed of two oval lamellae and a small ventral lobe. **Isodiplosis deuthera** sp. n.
- 7 Length 2 mm.; tarsal claws toothed on fore-legs only; third flagellar segment (fig. 38) with two whorls of circumfila united laterally
- Clinodiplosis leguminicola** sp. n.
- Length 1 mm.; tarsal claws simple on all legs; third flagellar segment with two whorls of circumfila not united laterally (fig. 44) . . . **Giardomyia britannica** sp. n.

Larvae

- Larva without sternal spatula, mouthparts strongly sclerotised (Pl. II, fig. 6), possesses pronounced pseudopods, a dorsal anus and three pairs of long anal setae (fig. 34), orange in colour, 2.5 to 3 mm. long. (*Predatory on other midge larvae.*) **Lestodiplosis trifolii** Barnes
- Larva with sternal spatula 1
- 1 Globular surface sculpturing present (as in figs. 9 and 10) 2
- Globular surface sculpturing not present 3
- 2 Sternal spatula lobes steeply triangular and quite pointed apically, stem length eight times its width in the middle and six times height of lobes (fig. 9); colour uniform pink. (*Feeds on seed of red clover only*)
- Dasyneura leguminicola** Lintner
- Sternal spatula lobes less steeply triangular and slightly rounded apically, stem length five times its width in the middle and six times height of lobes (fig. 10); colour yellowish, often with anterior fat bodies pink. (*Feeds on seeds of red, white and Alsike clover*) **Dasyneura gentneri** Pritchard
- 3 Anal segment with moderately long setae 4
- Anal segment without moderately long setae 5
- 4 Length 1 to 1.4 mm., colour pale yellow to white; anal segment (fig. 51) with two pairs of tubercle-based, long setae and two pairs of tubercle-based, short setae; sternal spatula with two moderately pointed lobes, the stem slightly wavy **Isodiplosis deuthera** sp. n.
- Length 2 to 2.5 mm., colour pale pinkish-orange; anal segment (fig. 40) with one pair of tubercle-based long setae, one pair of small, ventral, tubercle-based short setae and two pairs of well-developed tubercles with squat, rounded, apical setae **Clinodiplosis leguminicola** sp. n.
- 5 Adanal segment bearing setae (fig. 28), anal segment possessing an elongate anus and four pairs of terminal, tubercle-based setae, separated by a series of small conical projections of the epidermis; length 2.5 to 3 mm.; colour yellowish-orange with greenish internal spot; sternal spatula long and narrow with two rounded lobes (fig. 28). (*Predatory on aphids on clover*)
- Phaenobremia aphidivora** Rübsaamen
- Adanal segment without setae 6
- 6 Length 2 mm., colour yellow; anal segment bearing three pairs of tubercle-based short setae, one pair very small and setae of the largest pair short and conical (fig. 57); sternal spatula well developed with two clearly-defined, moderately pointed lobes **Contarinia** sp.
- Length 2.4 to 3 mm., colour yellowish-orange; anal segment bearing fine fingerprint sculpturing on ventral surface, and four pairs of tubercle-based, short, conical setae (fig. 21); sternal spatula well-developed with a long narrow stem and two pointed lobes broadly divided by a V-shaped emargination. (*Feeds on seed of red and white clover*) . . . **Tricholaba barnesi** sp. n.

VI. SUMMARY

A study of the gall midge (Diptera : Cecidomyiidae) fauna of clover heads has shown that there are three species, namely *Dasyneura leguminicola* Lintner, *D. gentneri* Pritchard and *Tricholaba barnesi* sp. n., whose larvae destroy clover seed in Britain.

Dasyneura gentneri and *D. leguminicola* are separated on morphological and biological grounds. The morphological criteria used are demonstrated to be valid in separating other closely allied *Dasyneura* species, namely *D. mali* Kieffer from *D. pyri* Bouché and *D. affinis* Kieffer from *D. violae* Loew.

Seven other gall midge species occur on clover heads in Britain. The larvae of two of these, *Phaenobremia aphidivora* Rübsaamen and *Lestodiplosis trifolii* Barnes are predatory on aphids and other gall midge larvae respectively. The feeding habits of the remaining five species, namely *Clinodiplosis leguminicola* sp. n., *Giardomyia britannica* sp. n., *Isodiplosis deuthera* sp. n., *Brachyneura squamigera* Winnertz and *Contarinia* sp., are not known, although it is suggested that larvae of *Isodiplosis deuthera* may feed on decomposition products or on fungus spores.

Illustrated keys are given for the identification of males, females and larvae of gall midges occurring on clover heads in Britain.

VII. ACKNOWLEDGMENTS

This work forms part of a thesis submitted to London University for the Ph.D. degree. The author wishes to express his gratitude to the late Dr. H. F. Barnes for his constant guidance and encouragement. He would also like to thank Dr. K. Mellanby and Dr. C. B. Williams for placing at his disposal the facilities of the Rothamsted Entomology Department, where the work was carried out, and the South African Department of Agriculture through whose generosity it was possible to make this study.

The photographs used for figures 2, 4, 5 and 6 on Plate I, and figures 3 and 4 on Plate II were taken by Mr. V. Stansfield of the Rothamsted photography staff.

VIII. REFERENCES

- ANON., 1954, *Canad. Ins. Pest Rev.* **32** : 305.
 BARNES, H. F., 1928, British Gall Midges II. *Lestodiplosis* Kieffer. *Ent. mon. Mag.* **64** : 68-75, 142-8.
 — 1929, Gall Midges (Dipt., Cecidomyiidae) as enemies of aphids. *Bull. ent. Res.* **20** : 433-42.
 — 1944, Gall Midge larvae living among stored seed. *J. R. hort. Soc.* **69** : 242-3.
 — 1946, *Gall Midges of economic importance*. Vol. II. *Gall Midges of fodder crops*. London.
 — 1953, The biological approach to the species problem in Gall Midges (Dipt., Cecidomyiidae). *Ann. ent. fenn.* **19** : 2-24.
 — 1954, Gall Midges living in White Clover flowers. *Entomologist* **87** : 258-64.
 BISHOP, G. W., 1954, Life history and habits of a new seed midge, *Dasyneura gentneri* Pritchard. *J. econ. Ent.* **47** : 142-7.
 COMSTOCK, J. H., 1880, in Report of the Entomologist for 1879. *Rep. U.S. Dep. Agric.* **1879** : 193-9.
 CREEL, C. W. and ROCKWOOD, L. P., 1947, The control of the Clover-flower midge. *Fmrs' Bull. U.S. Dep. Agric.* **971** : 1-9.
 EDWARDS, F. W., 1937, New records of British Cecidomyiidae (Diptera) with taxonomic notes on certain genera. *Ent. mon. Mag.* **73** : 145-54.
 FELT, E. P., 1908, Studies in Cecidomyiidae, II. *Bull. N. Y. St. Mus.* **124** : 307-442.
 — 1915, A study of Gall Midges, II. *Ibid.* **175** : 79-213.
 — 1920, A study of Gall Midges, VII. *Ibid.* **231-2** : 118-23.
 — 1939, A new gall midge of rhododendron. *J. N.Y. ent. Soc.* **47** : 41-42.
 FOLSOM, J. W., 1909, The insect pests of clover and alfalfa. *Bull. Ill. agric. Exp. Sta.* **134** : 111-98.
 FORBES, S. A., 1886, Notes on the past year's work. *Canad. Ent.* **18** : 176-7.
 GUPPY, J. C., 1952, Forage crop insects, Ottawa. *Canad. Ins. Pest Rev.* **30** : 70-71.
 KIEFFER, J. J., 1895, Die Zoocecidien Lothringens (VI. Fortsetzung). *Ent. Nachr.* **21** : 171-6.

- KIEFFER, J. J., 1909, Contribution à la connaissance des insectes Gallicoles. *Bull. Soc. Hist. nat. Metz* (3) 2: 1-35.
- 1912a, *Neue Gallmücken-Gattungen*. Bitsch.
- 1912b, Nouvelle contribution à la connaissance des Cécidomyies. *Marcellia* 11: 219-35.
- 1913, Diptera. Fam. Cecidomyiidae. In Wytsman, *Genera Insect.* 152: 1-346.
- LIEBEL, R., 1889, Dipterologischer Beitrag zur Fauna des Reichslandes. *Ent. Nachr.* 15: 282-6.
- LINTNER, J. A., 1879, The Clover-seed fly—a new insect pest. *Canad. Ent.* 11: 44-45.
- 1879, On *Cecidomyia leguminicola* n. sp. *Ibid.* 11: 121-4.
- MANSON, G. F. and Staff, 1946, Insects of the season 1945 in Alberta. *Canad. Ins. Pest Rev.* 24: 92-104.
- METCALFE, MARGOT E., 1933a, *Dasyneura leguminicola* (Lint.). The Clover seed midge. *Ann. appl. Biol.* 20: 185-204.
- 1933b, The morphology and anatomy of the larva of *Dasyneura leguminicola* Lint. (Diptera). *Proc. zool. Soc. Lond.* 1933: 119-30.
- NIJVELDT, W., 1954, The range of prey of *Phaenobremia urticae* Kffr. and *Ph. aphidivora* Rübs. (Diptera, Itonididae). *Ent. Ber.* 15: 121-11.
- 1955, Additional notes on my paper on the range of prey of *Phaenobremia aphidivora* Rübs. and *Ph. urticae* Kffr. (Diptera, Itonididae). *Ibid.* 15: 436-7.
- 1957, Aphid-eating Gall Midges (Cecidomyiidae), with special reference to those in the Barnes collection. *Ibid.* 17: 233-9.
- ORMEROD, ELEANOR A., 1891, Report of Observations on Injurious Insects and Common Farm Pests during the year 1890. *Fourteenth Report*, p. 23, London.
- PRITCHARD, A. E., 1953, The White Clover Flower Midge as differentiated from the Red Clover Flower Midge. *Pan-Pacif. Ent.* 29: 128-32.
- ROZSPAL, J., 1942, [Report on adverse factors affecting cultivated plants (with the exception of oats, potatoes and flax) during the vegetation period 1940-41 in Moravia.] *Ochr. Rost.* 18: 17-24. (Quoted from *Rev. appl. Ent.* (A) 35: 100-1; 1947.)
- RÜBSAAMEN, E. H., 1891, Ueber Gallmücken aus zoophagen Larven. *Wien. ent. Ztg.* 10: 6-16.
- 1912, Ueber deutsche Gallmücken und Gallen. *Z. wiss. Insekt-Biol.* 8: 97-102.
- 1917, Cecidomyiden Studien VI. *S. B. Ges. naturf. Fr. Berl.* 1917: 60-64.
- SAUNDERS, W., 1882, Insects injurious to Clover. *Rep. ent. Soc. Ont.* 12 (1881): 37-48.
- SCHØYEN, T., 1926, *Beretrn. Skadeinsekt. Planteskdyd.* 1924 og 1925, 8. (Quoted from Barnes, 1946.)
- STOKES, BARBARA M., 1954, Behaviour as a means of identifying two closely allied species of Gall Midges. *Brit. J. Anim. Behav.* 3: 154-7.
- THORPE, W. H., 1956, *Learning and instinct in animals*. London.
- TULLGREN, A., 1917, Skadejur i Sverige åren 1912-16. *Medd. CentAnst. Försöksv. Jordbr., Stockh.* 152: 84.
- WEHRLE, L. P., 1929, The Clover-Flower Midge (*Dasyneura leguminicola* Lintner). *Bull. Cornell agric. Exp. Sta.* 481: 1-35.
- WINNERTZ, J., 1853, Beitrag zu einer Monographie der Gallmücken. *Linnaea ent., Berl.* 8: 154-322.
- YATES, F., 1934, The analysis of multiple classifications with unequal numbers in different classes. *J. Amer. Stat. Ass.* 1934 (March): 51-56.

PLATE I

FIG. 1.—Breeding cage *in situ* on clover plant.

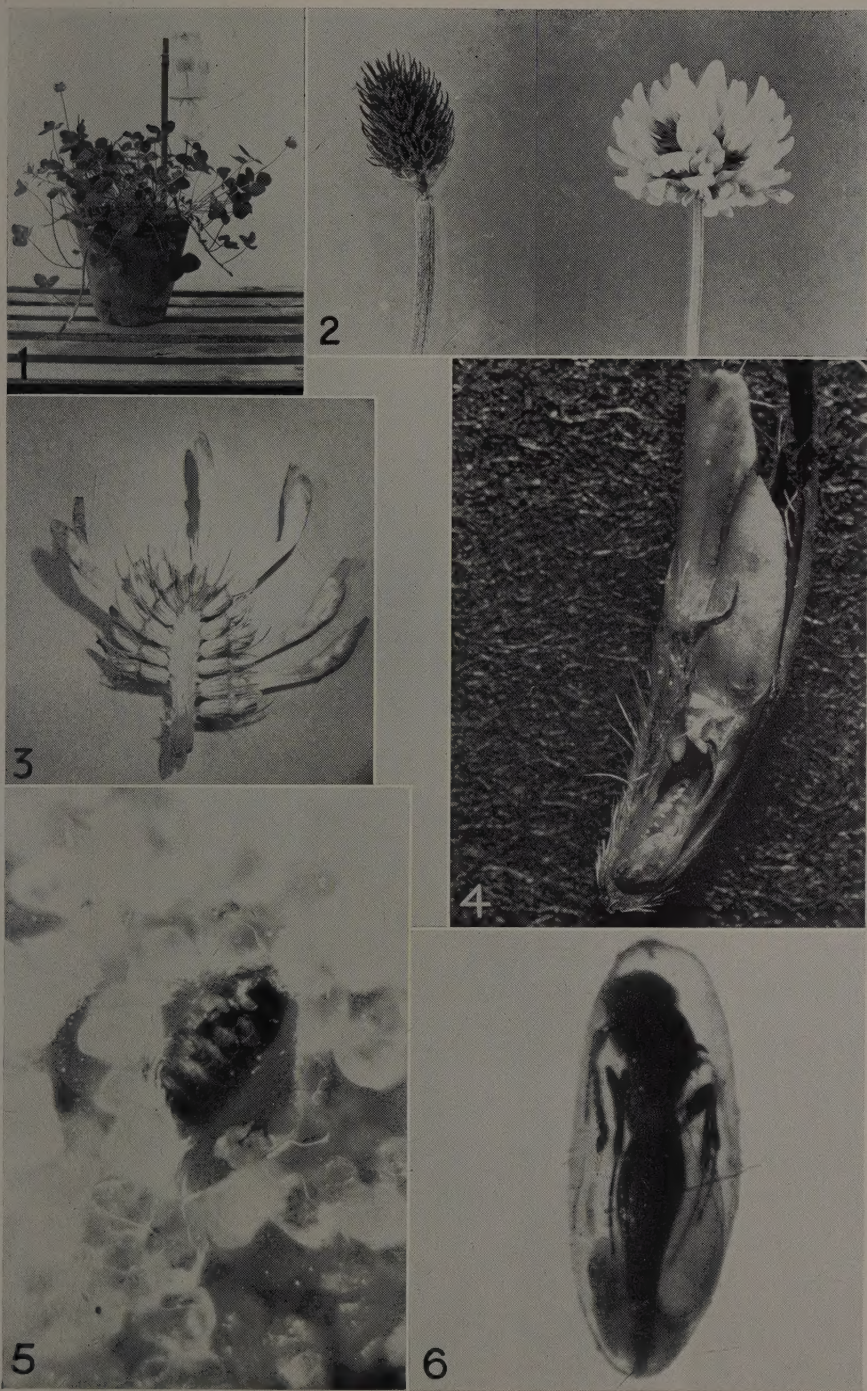
FIG. 2.—Left: green head of red clover selected for oviposition by *D. leguminicola*. Right: open flower of white clover selected for oviposition by *D. gentneri*.

FIG. 3.—Section of red clover head partially infested by *D. leguminicola* larvae. Normal florets are fully extended, infested ones stunted and swollen.

FIG. 4.—Floret of red clover with part dissected away to show a *D. leguminicola* larva beside the shrunken ovary.

FIG. 5.—Pupa of *D. leguminicola* in observation tube. The shed sternal spatula may be seen on part of the larval skin in the lower right-hand corner of the cocoon.

FIG. 6.—Parasitic Hymenopteron in larva of *D. leguminicola*. Sternal spatula of the larva may be detected at the apex of the larval skin.



Gall midges of clover flower heads.

PLATE II

FIG. 1.—*D. gentneri* inserting ovipositor between wing and keel petals of white clover floret to deposit an egg.

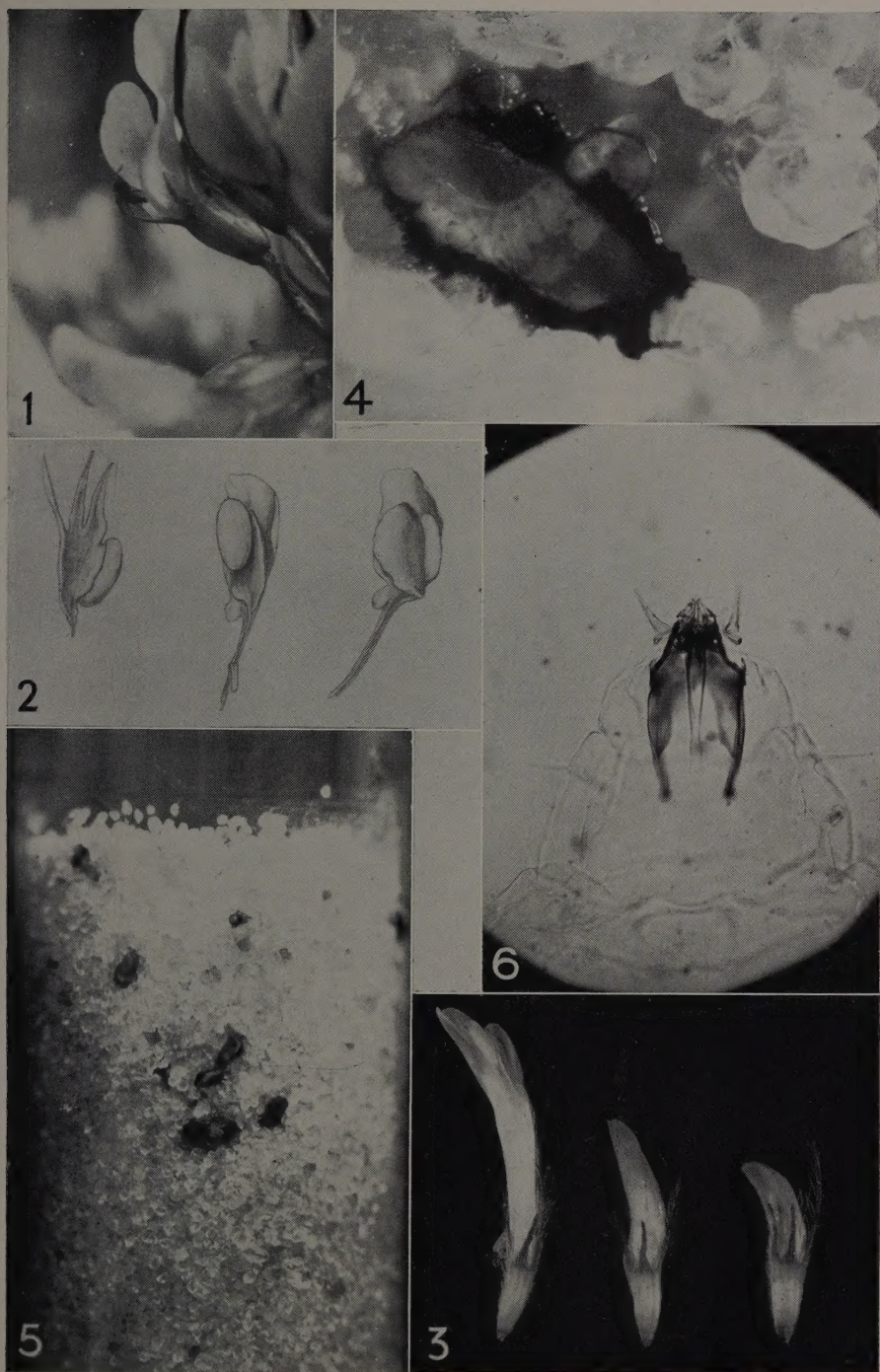
FIG. 2.—Parts of dissected white clover florets showing *D. gentneri* cocoons attached to the outside of the calyx, the inside of a keel petal, and between wing and keel petals.

FIG. 3.—*Left*: normal floret of red clover. *Right*: two stunted florets infested with *T. barnesi*.

FIG. 4.—*P. aphidivora* in black cocoon, $\times 25$.

FIG. 5.—Upper section of observation tube showing black *P. aphidivora* cocoons in white sand.

FIG. 6.—Mouthparts and endoskeleton of mature larva of *L. trifolii*.



Gall midges of clover flower heads.

